

nature*March 6, 1975*

Who makes the decision on life and death?

Thou shalt not kill; yet needst not strive,

Officiously to keep alive.

Arthur Hugh Clough's witty Victorian Ten Commandments for the comfortable sometimes have a very modern ring to them—none more so than the sixth when put in the context of modern medicine. Last week BBC television gave us two looks at spina bifida; the first was an hour-long documentary of great distinction which managed at once to educate, to convey a message of hope, to present the caution of scientists in an understanding light and to provoke on the question of whether severely handicapped babies should be left to die. The second took up the running on this last issue in the programme "Controversy", and Dr John Lorber, who is one of many pediatricians who give parents the chance to withhold treatment in bad cases, was confronted with two doctors and a theologian who believed this option should not be given, and an audience which seemed (at least after editing) to divide roughly equally; it's tantalising that the producers of "Controversy" don't ask for a vote, even after the formal ending.

It was no great surprise that the parents at the debate provided the most emotional and memorable contributions, nor that the lady from the National Secular Society should have said what she did about terminating more lives. Somewhat suprisingly, the theologian Professor Gordon Dunstan, stated the case for preserving life in muted terms: let them die and you reduce the capacity for heroism and the incentive to medical science. For the rest, the debate rested fairly inevitably on the technical data and philosophies of different doctors.

And yet one thing did emerge with remarkable clarity—that it is the doctor, not the parents who makes the decision whether the child lives or dies. If the doctor believes severe cases should be allowed to die relatively peacefully then he will be capable of convincing almost all his clients to follow this course. If, on the other hand, he believes severe cases deserve every attention that medical science can bestow, he will be able to take parents along with him in that direction.

There is nothing new in all this. In the matter of birth control and abortion, sophisticated women have recognised for many years the necessity for pre-selecting the person whom they consult.

The reason that the doctor has so much power in the decision-making process is not simply that he has immense knowledge where his client has none—after all, the decision that the parents have to make is one in which technical knowledge is only one ingredient. Rather,

the parents must be only too aware that if the doctor expresses a view one way and they choose to ignore his opinion, they have to live not only with their own decision but with the doctor himself for months if not years. It is presumably for the same reasons that people don't more often pick arguments with teachers, bosses and dentists.

Dr Lorber has broken valuable new ground by publicising a new option for parents so courageously (although the point was made in the programme that doctors do occasionally confront comparable problems in other circumstances). Many have, and will, object to the granting of the option on grounds that there are possibilities that the child could live a happy life, but it is striking how little public uproar there has been on grounds of morality; it must be that the obvious detached sincerity with which many parents have elected not to support their child's life, and the frequent damage to family life—even culminating in suicide—that spina bifida can bring, prevents some of the more conventional sanctity-of-life responses that one would otherwise expect. But how do we ensure that it is truly an option, and for that matter that the option is available to those that need it, regardless of the hospital area in which they happen to live?

However carefully the doctor may present both sides of the case it is inevitable that his clients are strongly influenced by the slightest hint of a preference one way or the other, and the choice which was originally theirs has subtly been surrendered to the doctor. In many cases this may be no bad thing, but this does not necessarily justify continuing a procedure in which some clients may eventually look back in some puzzlement at how they reached a particular decision. Offering a second opinion does not seem a good safety net; few seek it, and although doctors may claim this is because clients can come to their own conclusions, it may equally indicate tentativeness of clients in the face of medical men.

An idea worth consideration is that the medical profession, or even some organisation outside the profession, should provide a new sort of service to doctors and clients in such circumstances. Someone without any involvement in an individual case should be deputed by the doctor to present the options to the client, proffer advice if requested and then convey the decision to the doctor. In this way not only would it be clearer that the decision has been made by those who must ultimately bear the responsibilities, but also there would be assurance that the range of options discussed reflects all medical possibilities. □



Huxley remembered

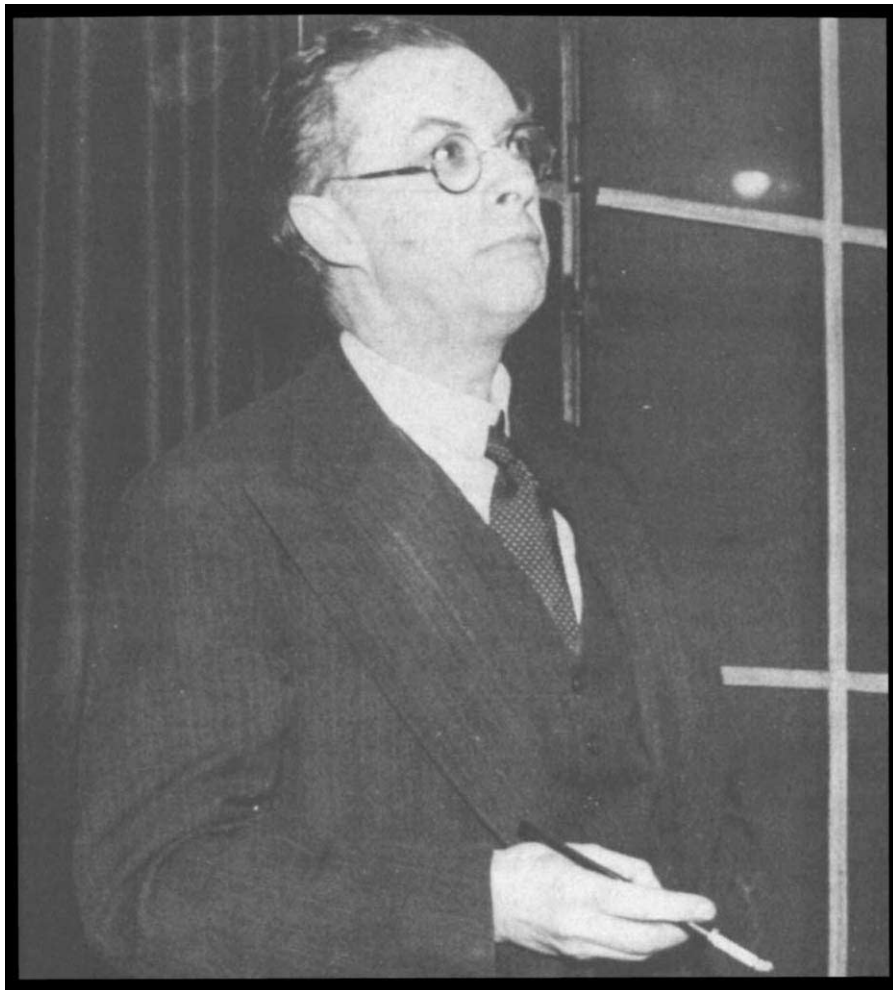
SIR JULIAN HUXLEY, FRS, one of the best known scientific personalities of our day, died in London on February 14 at the age of 87.

Huxley was a King's Scholar at Eton and went on to Balliol, Oxford, where he became Brackenbury Scholar and, from 1910–12, a lecturer in zoology. As an undergraduate, he was also awarded the Newdigate Prize for Poetry. At 25, he became Professor of Zoology at the Rice Institute in Houston, but at the outbreak of war returned home and joined the Army Intelligence Corps, serving at the Italian front when the armistice was signed. He returned to Oxford in 1919 as a Fellow of New College and Senior Demonstrator in Zoology, and in 1925 was appointed Professor of Zoology at King's College, London. He also held the office of Fullerian Professor of Physiology at the Royal Institution from 1926–28, but resigned both scientific posts to devote more time to writing and research. His association with the Zoological Society of London as Secretary began in 1935 and lasted until 1942, during which time he helped to develop Whipsnade Zoo. His lifelong interest in Africa resulted in his being a member of the committee of Lord Hailey's 'African Survey' from 1933–38, and a member of the 1944 Committee on Higher Education in West Africa.

Because of his experience and powerful interest in the problems of social evolution and education, the British government persuaded him to become the first Director-General of UNESCO in 1946.

His many publications on animal behaviour, evolution and genetics are too numerous to mention. His *Problems of Relative Growth* (1932) was a turning point in the study of differential growth of parts of the body, and *Evolution, the Modern Synthesis* (1948) remains the most comprehensive modern work on this subject. But beguiled audiences of the BBC's 'Brains Trust' programme will remember him best as one of the original panellists, and particularly during the Second World War. Huxley also lent his support to many other institutions—the British Humanist Association, the Family Planning Association, the Nature Conservancy, and so on.

Recognition of his work came from all sectors. Among many were the Kalinga Prize (1953) for popular science writing, the Darwin Medal of the Royal Society (1957) for contributions to the study of evolution, and a Knighthood in 1958. □



JULIAN HUXLEY was only a decade or so older, but he was one of my 'heroes' from the time (1921) when I was starting research in chemical embryology. I never knew him really well and never spent very much time with him, but he was always there as a kind of leading figure "amidst the encircling gloom"; and occasionally our lives impinged strongly on each other. In the 1920s he was perhaps best known for his work at Oxford on the hybrid control of amphibian metamorphosis. This was outside my field, as I never worked in classical endocrinology, but when the 1930s came I was extremely interested in Julian's book on heterogony (or heterauxesis, as it afterwards came to be called) *Problems of Relative Growth*. Together with Georges Teissier in France I spent a good deal of time in applying these conceptions to the relative growth of the chemical constituents of animal bodies. This turned out to be one of those lines of investigation which, though interesting in themselves, do not lead immediately to anything further. Quite different was the situation with Julian's *Elements of Experimental Embryology*, which he wrote with G.R. de Beer in 1934. This was at the beginning of the story of embryonic induction; based on

the fundamental experiments of Spemann and his collaborators, it set the stage for a vast amount of work on the 'morphogenetic hormones'. Terminologies change, and organisers today might be called 'semantophore macro-molecules', but although such great advances have been made in the study of the hereditary 'books of instructions', the nucleic acid chains, it seems that still today it has not been possible to identify the molecules which pass from cell to cell and determine specific differentiation destinies. Doubtless messenger RNA is in the picture somewhere. In any case Huxley and de Beer was a stirring overture to a field of experimental morphology still of the highest importance and fascination today.

But neither Julian Huxley nor I were spirits circumscribed by the walls of the laboratory. Both of us were interested in philosophy, religion, artistic creation, history and literature; both of us, like Charles Sherrington and many other scientists, were moved to write poems from time to time. We must retrace our steps, for *Religion without Revelation* came out in 1927; while my *Sceptical Biologist* was 1929. Of course our standpoints were rather different, though both in a way syn-

cretistic. Julian upheld the family tradition of agnosticism and what came to be called 'humanism', while I was for accepting the practice of all the forms of human experience while not granting absolute validity to any of them. Consequently I retained liturgical religion and was content to be described as a left-wing Christian socialist --but all this never came between us, and on a great many matters we saw closely eye to eye. It was rather like an experience I once had with Fred Hoyle. The Cambridge Union proposed to debate that "wherever science advances religion recedes", and he was to be the proposer, while I was cast as the opponent. So we had tea together, and concluded, after carefully looking into it, that there was just that hair's breadth of difference between us as to make the affair possible. It was rather like that with Julian and me.

But politics kept on breaking in. Over a couple of decades, centering on the 1930s, the "Younger Scientists" became a label as political as the "Young Turks" had been in an earlier time. Here the essential crux was the relations of science and society; what really were they in the liberal democratic, bourgeois, capitalist society that we all knew, what had they been in the societies of the long-past world, and what could they be imagined to be in socialist society, whether nascent as at that time in the USSR, or more ideally foreseeable in future socialist societies? This movement of the 1930s was the lineal ancestor of such bodies as the British Society for Social Responsibility in Science today; with the big difference that present leading men, such as Joseph Rotblat and Stephen Rose, are contending with a world of nuclear power and nuclear weapons in which the problems of pollution have come much more into the foreground than they were in the 1930s. But in those days you had a very powerful group, of which perhaps Julian Huxley and J. B. S. Haldane were the oldest, including Conrad Waddington, Desmond Bernal, Lancelot Hogben, Dorothy Crowfoot and Hymen Levy. Other scientists were radical, though not on the left, such as Cyril Darlington and John Baker, while others yet again, like Patrick Blackett and Solly Zuckerman, so phrased their sociological beliefs as not to lose touch with the 'corridors of power' and the establishment, thus retaining a chance of influencing national policy. All these 'elements', *quorum pars minimus fui*, met together from time to time in the "Quotes and Tots", a dining club in London which was only extinguished by the Second World War. Needless to say, it took its name from the phrase *quot homines tot sententiae*, but in fact

throughout the 1930s there was a real consensus of agreement among the "Younger Scientists". Their *pronunciamientos* constantly enraged the older generation entrenched in Burlington House, where so strong was the prejudice against scientists doing anything other than science, that I was reduced (though I do not think Julian ever was) to adopting a pseudonym for some of my extra-scientific writings. Some may remember that in allusion to certain extremely distinguished officers of the Royal Society, the "Younger Scientists" were accused of riding roughshod over hill and dale.

Subjects like animal behaviour and bird-watching were always closed books to me, a side of Julian that I never knew. Our orbits came into the closest conjunction, however, after the foundation of UNESCO in 1946. I always thought it was Ellen Wilkinson, not R. A. Butler as has recently been said, who nominated Julian Huxley as the first Director-General; at any rate I was far away in China at the time. I had been one of those who throughout the last years of the war had made great agitation to get science included with education and culture in this new specialised agency of the United Nations. And it was therefore the greatest of pleasures to get a cable from Julian (I forget whether it was in Chungking or Nanking) asking me to return at once to head up the Natural Sciences Division. I duly came, we established ourselves in a large, gaunt, empty house of ambassadorial character in Belgrave Square, and the recruitment of staff went merrily on. The UNESCO years brought me into very close touch with Julian and that was the time I got to know him best. In those days UNESCO officials were not bound down by too much red tape, as happened later; for example when the Naples Marine Biological Station was about to close down, as its sea-water pumps had worn out, a couple of telephone calls with Stafford Cripps elicited that there were plenty of American surplus pumps on the quayside in that very city, and these could be used to keep things going. Or again, as soon as we knew that the Poles had lost, during the war, all their printing machines capable of setting up mathematical equations, Julian and I by a couple of telephone calls could get some new machines moving on their way to Warsaw.

The UNESCO period, fortunately, found Julian Huxley in one of his periods of most frenetic energy and activity. Our daily conferences were unforgettable, with André Thomas Thomas for culture, and Kuo Yu-Shou for education. Dear Dr Kuo, I had known him a long time before when he was Commissioner of Education in

Szechuan Province. Those were the days when we formulated between us many a policy for which afterwards, indeed for the rest of his life, Julian Huxley did great propaganda work—the liquidation of illiteracy, the control of population increases, and the reproduction of great classical spiritual books in many languages other than those in which they were originally written. Some of these enterprises have prospered remarkably through the years—for example the international understanding inculcated by the *UNESCO Courier*, which appears all over the world in many languages; or again the *UNESCO History of the World*. Julian used to maintain that this was originally my idea, but I feel quite certain that it was his, and all I did was to back it up through thick and thin at various meetings, including those of the Executive composed of the delegates of all the member states. This work in many volumes must now be in school libraries all over the world and really may be said to be fairly free from nationalistic prejudices. Thomas, Kuo and I were very close to Julian, and only Thomas carried on for any length of time after the first change of Director-General. Besides, there were not only the meetings but also very companionable walks and meals on Sundays in the forests of Fontainebleau and Rambouillet where we could compare notes *ad lib*.

Finally, in a last act, as it were, Julian and I enormously enjoyed investigating Amerindian archaeology on the occasion of the Second General Conference of UNESCO in Mexico City. Together we scoured the wonderful National Museum, visited unforgettable sites, like Teotihuacan, Xochicalco and Chichen-Itza; and sat at the feet of Mexican scholars and writers like Alfonso Caso, Miguel Covarrubias and Alberto Ruz-Lhuillier. All this bore much fruit for me later on when in the fourth volume of *Science and Civilisation in China* I had to face the problem of pre-Columbian culture contacts across the Pacific from East Asia.

Julian Huxley was a man for whom nothing human was foreign; one thought naturally of the phrase *nihi humanum me alienum puto*. He was blessed beyond measure by his marriage with Juliette, happy essentially from beginning to end, right from the day when her father's *vignerons* took Julian to be Oexle—*le type qui a inventé les degrés*. All their friends think of her with the deepest affection, and hope that knowledge of this may moderate, even in however small a way, the sense of loss from which she, like us, is assuredly suffering. □

Joseph Needham

Huxley: the presence of the 'S' in UNESCO is largely due to him

JULIAN HUXLEY was the leading experimental biologist of his day at a time and in a place in which the ever more detailed validation of the concept of evolution was still thought of as the principal task of biology. The supplanting of comparative anatomy by what everybody came to call 'experimental biology' is a fascinating episode in the history of ideas. In the early days of the Darwinian revolution the elucidation of homologies and the working out of family trees had the same kind of appeal and confident self importance as molecular biology has today, and I know from personal conversations with him that E. S. Goodrich, the former Linacre Professor of Zoology and Comparative Anatomy at Oxford and the only British comparative anatomist of the same stature as Gegenbaur and van Wijhe, still regarded himself even quite late in life as a torchbearer and adventurous pioneer of the great new doctrine.

But, alas, comparative anatomy became an abuse and an impediment to progress, just as comparative physiology became in the next quarter of a century and as molecular biology will surely become in 10 or 20 years (for I foresee clearly a time when, regardless of what major problems in biology remain to be attended to, the sequencing of a protein will still be regarded as an intrinsically meritorious activity which will be taken to represent the courageous holding aloft of the banner of what was once a great revolution in biological ideas).

Because of his intellectual vitality and the great compass of his interest and understanding Huxley rightly earned for himself the position of acknowledged leader of the newer biology. Certainly nobody since has made contributions of comparable magnitude to fields so diverse as ethology (his papers on the courtship behaviour of the great crested grebe are acknowledged classics); physiological genetics; developmental physiology including the study of allometry or differential growth; and ecology, especially in relation to speciation.

And then again, Huxley was, as his grandfather had been, a great expositor of the notion of evolution, although sometimes his ideas on the subject were felt by his juniors, rightly or wrongly, to be wrong-headed—mainly because Huxley never really mastered the modern population-dynamical approach to evolution theory; and so great was Huxley's enthusiasm for the idea of evolution that he came in his later years to treat evolutionism as a sort of secular religion.

Huxley was a great tutor in the old

Oxford style, a man who, because he loved it, chose to teach the whole of his subject instead of teaching only the parts of it that interested him and for the remainder farming out his students to specialists in other fields. The influence of a really good tutor lasts through many generations of pupils, pupils' pupils and so on. It is pleasing therefore to reflect that through a lineage which can be worked out with names and dates in detail, Julian's son Francis was his own great grand-pupil. (The lineage is Julian Huxley—Gavin de Beer—J. Z. Young—P. B. Medawar—Francis Huxley.)

With these qualities of character it is not at all surprising that Huxley was kind and helpful to the young, for although people in his position are bombarded from all quarters of the Earth by manuscripts of which the authors profess to seek their recipient's candid opinion, Huxley bore with this kind of imposition very handsomely and often made the time to answer his correspondents at length—sometimes in his own handwriting.

I do not know and cannot imagine any scale of evaluation of scientific merit along which Huxley would not stand out as one of the foremost biologists of the 20th century. □

Peter Medawar

THE contribution that Julian Huxley made to the work of UNESCO in the field of science was remarkable and, in several respects, decisive.

In the first place, he fought to ensure that science was given a place among the organisation's concerns on an equal footing with education and culture. Thirty years ago, when the conference convened to adopt the Constitution of UNESCO was about to meet in London in November 1945, the issue was still in the balance. On one side, the followers of the classical humanist tradition thought it better to deal only with education and culture, since they both centred on the preservation and development of moral values. On the other, some scientists took the view that science had become far too complex as an intellectual and social activity, and too important on account of its practical applications, to be only one of the fields of competence of an institution; they looked for a distinct organisation solely and totally concerned with science. Huxley was one of those who checked these separatist tendencies. The presence of the 'S' in UNESCO is largely due to him.

Had the organisation restricted itself to dealing with education and culture,

it would have been no doubt easier to manage, with probably a greater immediate efficiency. But none of the difficulties encountered outweighs, in my opinion, the paramount advantages and significance of the existence of an organisation which embraces, through the inter-relationship of its various fields of competence, the comprehensive unity of the minds as a whole.

Once this inter-relationship of education, science and culture was adopted as the fundamental principle of UNESCO, no one could have been better qualified than Huxley, with his manifold gifts, varied experience and wide-ranging earlier ventures, to provide the framework of the new organisation's programme. And that was the main task to which he devoted the best of his energies and abilities, as Secretary-General of the Preparatory Commission (1946) and thereafter as the first Director-General (1946–48).

In the field of natural sciences, with the help of Joseph Needham, he devoted special attention to the restoration and reshaping, in collaboration with the International Council of Scientific Unions (ICSU), of the international scientific community which had been shattered by the war. But Huxley was no less interested in the social sciences, which he thought should have a part to play in all sectors of UNESCO's activities. Thus, he put an ethnologist in charge of the first 'fundamental education' pilot project in Haiti. His efforts to bring together scientists, educationists and users of the mass media in a wide-ranging movement for the modernisation of science teaching and, more generally, for the popularisation of science, provides another instance of his multidisciplinary approach to problems.

Science for him was not merely a body of knowledge and skills; it was the most advanced form of culture, the very basis of all values. This feeling of the close kinship of science and culture suggested to him the idea of a *History of the Scientific and Cultural Development of Mankind*. He put the proposal to the General Conference at its first session in 1946 and succeeded—though not without difficulty—in securing its adoption. And after he had relinquished his functions as Director-General he played a very active part in carrying it out as Vice-President of the International Commission which had the editorial responsibility for the work.

One final aspect of his contribution to UNESCO's science programme which deserves special mention is that he wished the organisation to foster among both governments and the

general public a better awareness of certain major issues affecting the future of mankind. With his biological background and his evolutionist approach, he was admirably equipped to understand such problems and put them in perspective. In matters concerning the preservation of nature and its ecological balances, the proper management of the resources of the biosphere, the quantitative and qualitative implications of population growth, and the problems of human settlements, the views and the programmes that Julian Huxley recommended to UNESCO were a quarter of a century ahead of the ideas of the time. They showed a remarkable perception of the true mission of international organisations, which we are now just beginning to discover. That mission is not simply to help member states in solving their particular problems. It is above all to bring the people of the world to understand the vital problems of mankind as a whole, which require for their solution a sense of togetherness and the joint efforts of the community of nations. □

René Maheu

By the death of Sir Julian Huxley we lose not only a distinguished scientist but a man with whom it was as easy to talk of art or literature as of biology. I met him first, I think, in 1921 in the exciting society at Garsington, where he and his wife were staying with Lady Ottoline Morrell.

At that time Huxley was a Fellow of New College and Senior Demonstrator in Zoology at Oxford. Though about as much archaeologist as biologist, I attended his course on genetics. In the practical classes which accompanied it we studied the inheritance of eye colour in a small superficially shrimp-like animal, a species of *Gammarus*. Chancing to make an unexpected observation which seemed to open up certain possibilities of general interest, I discussed the matter with him. In a flash he saw the point and greatly extended my ideas in our subsequent talks and in our delightful excursions to Plymouth to study the animal in its natural habitat and to obtain further material for our investigations. He, already a well known scientist, and I, an unknown undergraduate, at once started to research together. Our first account of that work appeared in *Nature* in 1925, and we published extensively on it in the next few years. And here I would stress a quality entirely characteristic of Julian Huxley. When we published our major article (in the *British Journal of Experimental Biology*), giving a detailed account of our results with the conclusions to be drawn from them, it appeared not under

the names of Huxley and Ford but of Ford and Huxley. It has been a lesson to me all my life: to encourage and give priority to my junior colleagues.

This seems to have been among the last pieces of experimental work in which Julian Huxley took part. His scientific writings, extending over many years, have been of fundamental importance; his success as Secretary of the Zoological Society of London, and as Director-General of UNESCO was outstanding and needs no encomium from me. I would, however, like to draw attention to his greatest and least recognised achievement.

In his position, he was frequently visiting universities and scientific institutes of various kinds. He would talk to those researching there and from this something unprecedented would emerge. He would encounter those who had, perhaps for months or years, been devoting their time to some biological problem of which, often enough, Huxley would know very little. In a short conversation he would almost invariably be able to throw a new light on it, and those who talked with him felt that his visit had been an outstanding occasion. It was a contribution to science of a most unusual and unselfish kind; one which only a genius could make. He obtained little recognition for it, but its cumulative effect was great and can never be assessed.

It is difficult to impart an idea of Julian Huxley's friendship. It can, perhaps, be comprehended in this. He would always speak of a friend better behind his back than to his face: few indeed deserves such a tribute. □

E. B. Ford

JULIAN HUXLEY was a great teacher, and not only in the early academic phase of his career. The extent of his influence on zoologists of my own and younger generations is not always realised.

He was a leader of the movement which gathered so much momentum in the early 1920s, away from conventional comparative anatomy and the construction of evolutionary family trees to experimental embryology, genetics, comparative physiology and functional analysis. In 1923, with Hogben, Crew and J. B. S. Haldane, he launched the Society for Experimental Biology and its journal, then called the *British Journal of Experimental Biology*; Hogben has described the quartet as the Founding Fathers of the society—and the society has done more to guide the development of biology and of young biologists in this country in its half century of thriving expansion than any other.

My association with Julian was closest in the late 1920s, when we col-

laborated with my father, H. G. Wells, in writing *The Science of Life*. This was conceived by H. G. as a companion to his earlier *Outline of History*, to set down plainly and clearly "everything that an educated man—to be an educated man—ought to know about biological science . . . We three got together in 1927 and we made a scheme that covered every division of our immense subject. We worked very harmoniously throughout and, after a part publication, produced the book in 1930." My quotations are from H.G.'s *Experiment in Autobiography*.

Those were strenuous years. Julian has written in considerable detail about *The Science of Life* in the first volume of his *Memories* (1970), and told how the harmony of the three authors was occasionally obscured by superficial dissonance. Most of the text was first written by Julian (who produced far the greater portion) and myself. H.G.'s functions were to edit what we wrote and to drive us on to write it. There were times of special stress in 1929, when the first of the fortnightly parts were published while others were in page proof, others in galley, others in typescript and some even in the planning stage. H.G. was never an easy man to work with, as many tempestuous episodes in his life reveal, and he could be furious when his collaborators failed to deliver their copy as soon as he wished, or wrote at much greater length than had been planned. But the storms soon subsided; in Julian's words "H.G. lost and recovered his temper, and so did I, but on the whole the atmosphere was gay and friendly". I am sure that the violence of H.G.'s storms was lessened, partly by his great respect for Julian's store of vivid and accurate information and partly by his realisation that he himself was being educated. He had studied under Julian's grandfather at the Royal College of Science; now he was learning from the grandson how far and how excitingly the subject had evolved since those days.

In any event, the book was written and widely read, appearing over the years in several editions and revisions and in several languages. In Julian's own assessment, "The work was indeed an important achievement . . . It is now out of print . . . but its effects are still manifest in the increased space allotted to biology in the educational curriculum, and the greater interest of the general public in biological facts and their consequences." What he does not say is that the work would never have seen the light had it not been for his enthusiasm, his great abilities, and, as I have hinted, his fundamental friendliness and generosity. □

G. P. Wells

international news

FUTURE historians of science may well record that a highly significant event took place in a California state park between February 24 and 27, 1975. Meeting at the Asilomar Conference Center here for three days of intense—and at times heated—discussions, 140 scientists from 16 countries agreed that strict controls should be placed on an exciting new technique which enabled genes from one organism to be transplanted directly into another.

This attempt at self regulation by a group of scientists is believed to be without direct precedent, for although restrictions have been imposed on research before, they have usually resulted from concern over the uses to which science will be put, or from public pressure. In this case, however, the call for controls has come from the very scientists most actively engaged in the research and it has come at the inception of a new field of study, before any known hazards have arisen.

Although the genetic transfer technique promises revolutionary advances in molecular biology, the conference decided that its use should be tightly controlled because the genetically modified organisms it creates may pose direct, but unpredictable, health hazards. Such concerns led a group of American biologists, headed by Paul Berg of Stanford University, to issue a call last July for a world wide suspension of research involving the technique, until the possible hazards associated with it have been evaluated (*Nature*, **250**, 175; 1974). This conference was called to try to assess the hazards and to decide under what circumstances such research should be resumed.

The meeting concluded, in short, that some genetic manipulation experiments could go ahead immediately, provided fairly strict safety precautions are adopted, but that others should not be attempted until safer procedures have been developed. It was also agreed that there are some types of experiments which are potentially so hazardous that they should not be carried out under any circumstances. A statement of the principal conclusions reached at the meeting is now being drawn up.

It was remarkable that even a statement of general principles was approved by all but a tiny handful of participants at the conference, for at times it seemed inevitable that there would be a disastrous split between those who

Berg conference favours use of weak strains

from Colin Norman, Pacific Grove

wanted to press ahead with their experiments and those who were arguing for restraint. In the end, however, the rift was healed by the realisation that safer procedures could be developed very rapidly to allow virtually all the planned experiments to proceed, so that any continuation of the suspension of experiments would be short lived. The nub of the matter is that gene transfer experiments could be carried out much more safely using biologically disabled microorganisms which would be unable to survive outside the laboratory, instead of the organisms that are commonly used in genetic research. Fortunately, it was generally agreed that by using what one participant described as "old fashioned steam genetic engineering", such enfeebled strains could be developed and produced in quantity in a matter of weeks, so that even the most gung-ho researchers were able to accept that it would be prudent to delay some of their riskier experiments until safer organisms are available.

Coming as they do from the people who will be most affected by them, the proposed controls on such experiments may seem like the classic case of the fox being set to guard the chickens—in fact, they have already been criticised as such by a group of radical scientists known as Science for the People. But the recommendations are much stricter than might be expected and, if implemented, they would require expensive modifications to many laboratories hoping to conduct gene transfer experiments. They are also more stringent than recommendations published last month by a committee of British scientists, chaired by Lord Ashby, which concluded that although development of safer strains of experimental organisms would be desirable, present safety precautions would probably be sufficient to allow most experiments to proceed.

The technique in question, according to Sydney Brenner of the MRC Laboratory for Molecular Biology, Cambridge, "is going to generate the most

exciting period, I think, in biology, and it is going to last for at least 10 years, maybe more". It involves use of a newly discovered class of enzymes, called restriction enzymes, to snip fragments of genetic material (DNA) from one organism and splice them to the DNA of another organism, such as a virus or bacterium.

The key to the technique is that some restriction enzymes sever one strand of the double-stranded DNA leaving what are known as 'sticky ends'. Two DNA molecules cut by the same enzyme will have identical ends, which can be joined together and annealed into a single molecule. Three types of experiment using this method of cutting and splicing together DNA fragments from different sources have either been carried out or are being contemplated. The first involves insertion of 'foreign' DNA fragments into a package of DNA called a plasmid, which can replicate inside a bacterium independently of the bacterium's chromosomes. A second type of experiment involves splicing fragments of foreign DNA into the DNA of viruses called bacteriophages, which attack and replicate in bacteria. And a third possibility involves joining together pieces of DNA from two different viruses, to form a hybrid virus.

The scientific promise of the technique is that it will allow fragments of DNA which are believed to be responsible for initiating specific chemical reactions to be inserted into a bacterium and copied every time the bacterium divides. The bacterial culture would then produce large quantities of the foreign DNA fragment, which could be studied more easily. The technique also offers the possibility of taking particular genes out of their normal environment and studying how they operate in the simple genetic system of a bacterium. And the construction of hybrid viruses may help to shed light on why some viruses cause tumours in animals.

In addition to that potentially rich scientific harvest from such genetic manipulation experiments, the technique may offer the more remote possibility of isolating genes which code for nitrogen fixation in leguminous plants and introducing them into the cells of crops such as cereals. If that could be accomplished the resulting hybrid cereals would no longer need a constant supply of nitrogen fertiliser. Another widely touted possibility is

that it may be possible to mass-produce the genes responsible for promoting the synthesis of insulin by growing them in bacterial cultures.

But the technique also involves the possibility of significant health hazards. The essence of the matter is that by combining fragments of DNA from different sources an infectious organism might be produced endowed with unpredictable biological properties. By splicing genes from two viruses, for example, the host range of the virus might be extended, so that a virus which does not normally infect might inadvertently be made to do so. The consequences of transferring segments of DNA from animal tumour viruses to viruses which commonly infect man, for example, could be disastrous. The chief reason for concern with such experiments is that segments sliced from a molecule of DNA by a restriction enzyme contain considerable amounts of genetic material in addition to the particular gene which is being studied, and the effect of that unknown DNA cannot be predicted in advance.

Another cause for concern is that the bacterium most commonly used in genetic research is a laboratory strain of *E. coli*, a bacterium which is normally present in the human gut. Although Dr E. S. Anderson, of the Enteric Reference Laboratory, Colin Dale, presented evidence suggesting that the particular strain of *E. coli* used in laboratory research does not survive for long in the gut in competition with other strains, it was universally agreed that more reliable safety factors are needed.

That realisation led to perhaps the most productive session at the conference—a session during which specific mutations were discussed which could be built into laboratory viruses and bacteria used for genetic research, to ensure that the organisms would be incapable of surviving outside the medium in which they were cultured.

According to Wacław Szybalski, of the University of Wisconsin, such biologically enfeebled strains could be produced “by the time we have slept off this meeting.” In short, the mutations would involve making bacteriophage lambda (the most commonly used bacteriophage for genetic manipulation experiments) unable to survive at human body temperature, unable to grow the tails needed to make it infectious, and able to infect only the particular modified strain of bacteria used in the experiment. *E. coli* could also be equipped with genetic mutations which would make it unable to synthesise diaminopimelic acid (DAP), a constituent of cell walls, so that it would have to be provided with DAP in order to survive. *E. coli* could also be made temperature-sensitive.

With the promise that such disabled organisms could be produced and made widely available in a matter of weeks, much of the concern about delaying experiments, which had been voiced during the first two days of the meeting, was considerably diminished. Consequently, a draft statement, drawn up by the organising committee and discussed on the final day, was accepted by an overwhelming majority of the participants, even though it called for many important experiments to be put off until safer laboratory strains of bacteriophage and *E. coli* are developed.

The draft statement outlined three levels of safety precautions which should be accompanying various genetic manipulation experiments. Only the lowest risk experiments should be carried out with existing strains of bacteriophage lambda and *E. coli*, the statement suggested, but they would require safeguards such as autoclaving all cultures, safe pipetting procedures and so on. Such experiments would consist of gene transfers between organisms which normally exchange genetic material—in other words, no novel genetic combinations would result from the experiment—and the insertion of genes from invertebrates and cold-blooded vertebrates into bacteria.

The next level of experiment—those embodying moderate risk—would require use of biological safety cabinets, negative pressure in limited access laboratories and use of some protective clothing. Estimates of how much it would cost to equip a laboratory to conduct such experiments varied widely, but it was generally reckoned that at least \$20,000–40,000 would be needed. In addition because physical safeguards cannot guarantee absolute protection against the spread of biological agents, such experiments should await the development of enfeebled strains of viruses and bacteria, the statement suggested. Experiments such as the splicing together of DNA from viruses, linkage of viral DNA to bacterial plasmids, and the joining of fragments of DNA from warm-blooded animals to bacterial DNA would fall into this category.

The highest risk experiments which could be done would again require the use of enfeebled strains of bacteria, and they should only be conducted in special laboratories equipped with airlocks to isolate them from other areas. Protective clothing should be worn, and showers would be required on entering and leaving the laboratory. Examples of such experiments are the fusion of genes to bacterial DNA when the resulting organism is likely to produce an agent toxic to the host, and work on viruses such as smallpox, which present a high risk in themselves.

Finally, after much debate, it was agreed that there are some types of experiments which should be ruled out altogether. Although such experiments were not defined, they would include the insertion into *E. coli* of the gene which specifies production of botulinus toxin. There would, however, be little justification for carrying out such experiments, except to produce lethal agents for biological warfare.

A statement setting out those general principles was agreed to by all but about three or four participants. Among the dissenters were two Nobel prize-winners, Joshua Lederberg, of Stanford University, and James Watson, of Cold Spring Harbor, both of whom had consistently challenged some of the basic assumptions underlying the conference.

Lederberg, for example, said in a statement he distributed at the conference, that “research on recombinant DNA is, in my opinion, the central way in which molecular genetics can contribute to the solution of important medical problems” and he warned repeatedly that any delays in carrying out the research should take into account the fact that important benefits may also be delayed. He also suggested that regulations governing such research are likely to be frozen in place, so that it would be difficult to make them less stringent later if it turns out that the risks have been overestimated. Lederberg also took exception to the classification of experiments into only three classes, pointing out that most of the contemplated studies would fall into the low or moderate risk categories, but the difference between the two is “considerable reconstruction of a laboratory”.

Watson drew attention to the discrepancy between regulations being proposed for genetic manipulation studies and those governing work on tumour viruses. “As someone in charge of a tumour laboratory”, he said, “we are working with something I feel is instinctively more dangerous than anything I have heard here”, yet the regulations governing research on tumour viruses are relatively lax. “I think you should just use common sense”, he said, but added that “you will have to live with the fact that somebody may sue you for \$1 million if you are careless”.

Underlying much of the discussion of the need for self-imposed controls was acceptance of the fact that science has lost much of the public support that it once enjoyed, and that if regulations were not imposed from within, legislation could be anticipated which would probably turn out to be much more restrictive. Whether this unprecedented attempt at self regulation will work, however, remains to be seen. □

Facing Israel's water problems

from Kapai Pines, Jerusalem

IN spite of a 25-year-old development scheme for water resources, Israel is faced with a crisis which threatens to damage seriously the country's agricultural programme. The Israelis' ingenuity in water management has achieved spectacular results, particularly in the cultivation of the so-called wilderness areas, but now the country has reached the point where exploitable resources are strictly limited, while consumption is growing and promises to go on growing.

Israel has a uniquely uniform pressurised supply system which encompasses virtually the whole country and integrates almost all of its water resources; 95% of available natural sources are used. The problem is how to expand, and even to conduct, the system in a situation of growing demand and limited supply, exacerbated by the problem of deteriorating quality.

Recently, water problems have been the subject of a lively public debate, in which almost everybody is trying to participate. In a memorandum submitted to the Prime Minister, Mr Rabin, the Minister of Agriculture, Mr Ozan, appeals for an urgent allotment of IL 500 million for the development of new water resources this year. Mr Ozan claims that there has not been any development of such new resources for many years and that, if immediate steps are not taken it will be necessary to divert 300 million cubic metres of water from agriculture in order to meet the expanding demands

of urban consumption. As a result, agricultural production will shrink by 25%.

Further, on February 10, almost all the Knesset (Israeli Parliament) factions united in adopting a resolution calling for water planning funds to develop new water resources while carefully utilising the existing resources to the peak of their economy and efficiency. The resolution warns against pollution of underground water, Lake Kinneret (Sea of Galilee), rivers and wadies; it calls for the implementation of sea water desalination by nuclear energy.

It is characteristic that the experts are divided among themselves as to the right solution. In the face of almost fully utilised natural resources what are the alternatives? There are several. There is, of course, the possibility of limiting the growth of consumption and adjusting it to the level of supply. It is also possible, some experts claim, to increase the actual yield of the resources by using water which has not been used for various reasons so far (brackish water or sewage effluents, very deep borehole well water or flood waters in winter streams). It is also possible to produce 'artificial water' (artificial rainfall and converted saline water). An additional way is 'mining' of water from underground reservoirs, which are one-time-only reserves.

As far as desalination goes, almost all experts agree that the technologies available at present are generally too costly for producing water for agricultural use. Since the urban and the industrial sections have been given priority, agriculture must take the blow. □

Mr. Wilson's deal with Russia

from Vera Rich

THE recent visit of Mr Wilson to the USSR has resulted, *inter alia*, in an impressive programme for scientific and technological cooperation between the UK and the USSR for the next decade. This programme is, in fact, a consequence of a number of meetings and discussions on this theme, going back as far as 1968 and culminating in the Agreement on the Development of Economic, Scientific, Technological and Industrial Cooperation signed in London on May 6, 1974.

The programme envisages a wide range of activities in which scientific and technological cooperation can be "encouraged" on a "mutually advantageous basis", ranging from high temperature plasma physics to "continuous beer production", and it is difficult to assess how far any particular clause will be implemented. Some subjects seem to have been included on a rather unilateral basis—it is difficult to see, for example, what precise benefit the UK would receive from the joint study of irrigation—and others are already there as a sop to the prevailing trends of world opinion (energy-saving studies and reduction of diesel exhausts); yet others (such as astronomy) are so wide ranging that it is difficult to know precisely what cooperation is envisaged. To complicate the matter further, the official programme blandly states after an impressive list of fields of cooperation that it is only "of a recommendatory nature, and is subject to modification and adjustment as and when necessary", a statement which appears to be something of an escape clause.

Nevertheless, some areas of cooperation can be defined more closely. A general statement of cooperation on "flameproof equipment for use in mines subject to sudden gas emissions and coal and rock bursts" seems to link up with a clause in the detailed schedule on "Cooperation to equip Soviet mining machines supplied to the United Kingdom with British-made electric equipment in accordance with British safety standards" (a somewhat surprising statement in view of the frequent *Novosti* releases on the safety of the Soviet mining industries) and the suggestion that the Soviet Union should participate "in the realisation of the programme of the National Coal Board for the modernisation and expansion of coal output" (which could raise some piquant problems with the Trade Unions). Meanwhile, in the Soviet Union, British know-how is to be used in the supply of goods ranging from "airport equipment" to "equipment for

Water yield and consumption in Israel (in millions of cubic metres per annum)

(a) Water yield

Resource	Potable water		Marginal water (brackish flood water reclamation of waters, sewage effluents)	
	Present yield capacity (1972-73)	Estimated future development (end of century)	Present yield capacity	Estimated future development
Lake Kinneret (Sea of Galilee)	560	150		10
Main aquifers	960	20	160	20
Floods	30	20-40	13	8
Sewage Effluents		100	26	100
Desalination	2	12		
Total	1,552	302-322	199	138

(b) Water consumption

Sector	Potable water		Other waters: brackish, flood-water, sewage effluents
	Consumption in 1972-73	Recent annual increase	
Agricultural	1,175	20	167
Domestic	285	12	
Industrial	60	5	32
Total	1,520	37	199

THE Indian University Grants Commission (UGC) has decided that emphasis during the Fifth Plan will be on consolidation and strengthening of existing university departments. Because of severe financial constraints, the UGC has asked all universities to curtail expenditure and limit their programmes according to its guidelines. Accordingly, the universities have been asked to formulate detailed programmes for development of existing teaching and research departments; introduction of new areas of specialisation in existing departments and setting up of individual departments; measures such as updating and modernisation of courses, and giving specific orientation to research activities; improvements in library facilities and services and general amenities for students and staff, such as study centres, hostels, residential quarters and health centres. The universities have also been asked to see that, while they plan new programmes, those initiated during earlier Plan periods are properly implemented.

At the undergraduate level, the UGC proposes to allow little or no further expansion in enrolment during the Fifth Plan, at least for formal, full-time studies. Expansion, if any, will come about only through part-time evening classes or correspondence courses. It has also been decided that the affiliated colleges would receive UGC assistance during the Plan, primarily for strengthening their faculty, improving their library and laboratory facilities, and installing workshops and related facilities. The colleges have also been advised to give priority to re-orienting their courses so that they become relevant to local, regional and national needs and emphasise utilisation of available natural resources.

● The UGC report for 1972-73, analysing the emerging problems and perspectives of higher education in India, blames unrest among students on the present system of examination, obsolescence of university curricula and the lack of good social life in colleges and universities.

The report points out that enrolment in university-level courses has left growth rate of the national economy way behind, resulting in lower per capita investment in higher education and also deterioration in standards of institutions where facilities are being

stretched to breaking point in accommodating increased student populations. (In 1972-73, there were 35.44 lakh students enrolled in 4,153 colleges in the country.)

Referring to growing unemployment among the educated, especially women and those who graduate from the universities in sciences and various professional courses, the report says that

Educating India

from Narender K. Sehgal, Jullundur.

much undergraduate education is not relevant either to the abilities and aptitudes of students or to the needs of the nation. With a very high failure rate at first degree level (about 50%) and large proportions of students being placed in the "third division" (aggregating less than 50% in an examination) at the postgraduate level, the report feels that "the present system of higher education is generating much waste and stagnation" and that "this raises serious doubts about the well being of the university in India."

The report further points out that the spread of higher education has not been very even, both area-wise and population-wise. Neither backward areas, nor students from these regions, have been able to get their fair share of facilities or opportunities for higher education — meaning that disparities have grown worse.

The commission blames on paucity of funds its inability to play a role in developing a system of higher education best suited to the genius of the people and the development of the country. It proposes a dialogue with the universities with a view to determining what can be done during the period of the Fifth Plan to make higher education a "little more meaningful and ultimately a fit instrument of change and development."

● The University Grants Commission has proposed 'radical' reforms in the present examination system. Meerut University, in Uttar Pradesh, is likely to be the first to try out the reform on an experimental basis during the next academic year. Similar experiments are

also expected to be launched in universities in other states.

Central to the proposed reforms is what is called a 'question bank'. Such a bank will have up to 100 questions which will be published and made available to both students and teachers at the beginning of the academic term. Seventy-five per cent of the questions asked in an examination will be taken out of the bank and the rest (25%), mostly numerical problems, from outside. The curriculum and the question bank for each course of study will be framed by Boards of Studies to be constituted for the purpose.

Also proposed is a card system for the examination questions. The cards containing questions, to be answered by individual examinees, will be picked up using a random process from a collection. (This will hopefully help reduce the use of unfair means at examinations—a very prevalent feature at present.) The candidates will be able to appeal to a committee if, on receiving the evaluated answer books, they feel dissatisfied with the evaluation. The committee will consist of the college principal, the teacher for the course and a representative of the students.

A university will be free to cancel a student's admission if he/she gets a 'D' grade in all his/her examinations and assessments. The grades A, B, C and D will replace the 'marks' being awarded in evaluations at present.

Universities as well as colleges will be required to conduct sessional or continuous assessments of students which will be shown on their grade sheets separately, as this will be aimed at measuring their "essential abilities". The name of colleges will also be mentioned on the degree/diploma of a qualifying student. The performance assessment of students will be required to be made over well distributed intervals of time.

Meanwhile the Union Education Minister recently told a meeting of the consultative committee attached to his ministry that the present examination system had collapsed completely and that reforms in this direction were urgently needed. He informed the members that 11 out of the 12 universities selected for launching examination reforms suggested by the UGC had already set up special implementation committees for the purpose. □

the production of tufted carpets".

Cooperation in the field of raw materials likewise ranges from window glass and timber products to "the enrichment of uranium in the Soviet Union from the raw material of British customers".

In general, the technological section of the programme tends to become

bogged down in detail, and the order of the items would surely form a fascinating study for the psychologist (cars, for example being listed immediately before toys). There is, however, a general overall stress on computer hardware and software, power production, including atomic power stations, heavy ridge group of exposed sediments. □

Wrong skull

THE skull illustrated on page 578 (February 20) was in fact KNM-ER1470 found at East Rudolf in 1972. It has been assigned to the genus *Homo*, sp. indet., and was found during prospecting in area 131 on the Karari industry and means of transport. □

SCIENCE and technology are receiving a new emphasis in the Arab world, partly as a result of the increased wealth of the oil-rich countries. The new source of wealth has made possible educational and research facilities, new institutions and programmes hitherto out of reach for most of the countries in the area. The first Conference of Ministers of Science and Heads of National Science Policy Organisations of the Arab States was held in Baghdad last year, and an Arab League Educational, Cultural and Scientific Organisation (ALESCO) has been formed in recent years to promote Arab culture, including science and technology. A number of Arab states have formed—or are forming—science policy machinery and programmes, emphasising applied research.

Egypt has traditionally been the most advanced Arab nation in science, and has built up a large pool of professionals who are much in demand in other Arab states. Iraq, which today has an ambitious five-year science plan, takes a distant second place. But while Egypt is poor, Iraq is now rich, and any promising research project can obtain funds there. Yet, ironically, Iraq is poor in personnel.

Trained personnel, in fact, are the main need of the Arab countries generally. Countries relatively rich in scientific manpower—Egypt, Lebanon and Jordan—are supplying the rest with personnel. Countries like Kuwait and Saudi Arabia are busy building laboratories and universities and advertising openings for staff.

Because of the wide differences in resources between the Arab states, Dr Ibrahim R. Shimi, head of the chemistry department in Ain-Shams University, Cairo, has proposed that a research council be established in the League of Arab States to which member states should allocate a proportion of their GNPs. This council would set up specialised research centres dealing with problems common to the area as a whole, such as cultivation of arid lands and desalination of sea water.

The Arab countries' experience with science policy formulation is varied. Egypt's has been complex, and began with its Science Council, established in 1956. The Science Council produced a national research plan for the universities, the new National Research Centre laboratories and the specialised institutions belonging to the ministries.

This council was superseded in 1961 by the Ministry of Scientific Research. In 1965, the Supreme Council of Scientific Research came into existence—but it was short lived and was replaced by the Ministry of Scientific Research in 1968. The Ministry in turn was replaced in 1971 by the Academy

of Scientific Research and Technology. The president of the academy, at present, Dr A. Abou-El-Axm, has ministerial rank and is responsible to the Prime Minister.

The academy not only formulates science policy but supports research and technology through grants. It also coordinates major projects, participates in development of science curricula, supports scientific conferences, and organises scientific publications and the popularisation of science, among other things. In addition, it is responsible for a number of research institutions in such areas as atomic energy, oceanography, petroleum and metallurgical research, and research on bilharzia.

The Arab World science revival

from David Spurgeon



Grain sorghum research, Lebanon

By comparison, Lebanon's history of science policy has been uncomplicated. It began in 1962 with the establishment of the National Council for Scientific Research (NCSR), and this single body not only was the first science policy-making body, but has continued as the sole one. The situation is further simplified by the fact that the same body that formulates policy also sees that it is carried out.

Lebanon went about its science policy planning deliberately and directly. The underlying assumption was as stated in its Five-Year-Plan for the Organisation and Development of Scientific Research in Lebanon:

"Lebanon is a small country with limited human and material resources. Its aspirations, whether for a higher standard of living or an enhanced cultural standing, can therefore only be conceived and implemented through utilisation of its resources, human and material, at the maximum possible

efficiency."

The NCSR therefore went about setting up the national science plan by first asking the Ministry of Planning what the country's overall objectives were. It promptly received a reply listing: maximum regular increase of the national income; equitable distribution of the national income among citizens; full employment; establishment of an equilibrium among the various economic sectors; and rational distribution of the population over the territory of Lebanon.

Eighty per cent of the plan deals with oriented research according to priorities carefully and simply laid out; 20 per cent deals with non-oriented or basic research. Themes accorded top priority are Lebanese climate, conservation of the natural milieu, health and disease in Lebanon, housing, and what are called "the essentials of productivity"—Lebanese raw materials, soil and water, and marine resources. NCSR's 1973 budget was 5,250,000 Lebanese pounds.

Some examples of the projects funded by the NCSR illustrate the strongly practical philosophy behind the plan: development of a high-protein, vitamin and mineral biscuit for low income groups; the study of Lebanese plants used in folk medicine with the aim of isolating and analysing their active elements with a view to their exploitation or synthesis; the industrial production of fish and molluscs using recycled human wastes; and research on processing of surplus fruit crops.

Joseph Naffah, Secretary-General of the NCSR, says that the practical orientation was adopted partly to attract attention to the accomplishments of science and to improve the climate of research, because in Lebanon, "the scientist is not respected in the sense that the self-made man is respected".

There is also a surprising amount of attention being paid to the popularisation of science. Salah Gelal, science editor of Cairo's newspaper *Al Ahram*, heads a staff of 12 science reporters and, with the financial backing of his newspaper, organises science clubs and fairs for Arabic youth. Last year, in Baghdad, UNESCO and the Union of Arab Broadcasters sponsored a meeting on the popularisation of science, and training symposia have been held and attended by representatives of a number of Arab countries. In fact there seems to be a growing realisation that the real costs of the area's long involvement in war include not only lost lives and material waste, but also a retardation of national development. The Arab states see a revival of science as essential to the fulfilment of their nation's potential. □

correspondence

Visa problems

SIR,—In your issue of December 13, Miss Peller gave an account of her experiences in trying to attend the Ninth FEBS Meeting in Budapest last August. As Chairman and Secretary-General, respectively, of the Federation of European Biochemical Societies at that time, we naturally regret that Miss Peller was unable to obtain a Hungarian visa in time, although we have been assured by the organisers of the meeting that a visa was in fact available, unknown to her, in Vienna three days before the meeting started.

Because there are no diplomatic relations between Hungary and Israel it was anticipated that it would be difficult for biochemists in Israel to apply for Hungarian visas. This problem was discussed during a visit to Budapest in February 1974 by the then Secretary-General of FEBS and Professor S. G. Van den Bergh, the FEBS meetings adviser. In consultation with the organisers of the FEBS meeting (the Hungarian Biochemical Society and the Hungarian National Academy of Sciences) a special procedure was worked out which enabled Israeli biochemists to send their passports some months before the meeting by diplomatic mail to London, where the FEBS Secretary-General obtained Hungarian visas on their behalf within 24 hours and then returned the passports and visas to the applicants in Israel, again by diplomatic mail. Though somewhat elaborate, these arrangements worked satisfactorily in the case of applications received in good time through the Israeli Biochemical Society. They did not, however, cater for biochemists like Miss Peller, who were not resident in Israel at the time and therefore applied directly to Hungarian consulates.

In Miss Peller's case there is at present a fundamental discrepancy between the information she received at the Hungarian consulate in Vienna where she was told she was on a "black list" and would not be admitted into Hungary and telegrams she received by one of us (H. R. V. A.) from the meeting organisers some days before the meeting started stating that her visa had been granted. Although we have not yet been able to resolve these apparent contradictions, we believe that a genuine effort was made both by the organisers of the FEBS meeting and the authorities in Hungary to establish a satisfactory procedure for dealing with

all visa applications and there is no evidence to suggest discrimination in the issue of visas.

Nevertheless, we think that there are several important lessons to be learnt by the organisers of future international conferences, as well as by intending participants. Principally, there is a need to ascertain at an early stage of planning a meeting the precise official regulations of the host country concerning visa applications, to include this information in the circulars sent to all intending participants, to make and publicise special arrangements for citizens of countries which do not have normal diplomatic relations with the host country, and to urge participants to apply early for visas. Finally, we believe it would be useful, particularly in the case of large conferences, to appoint a member of the organising committee to be responsible for dealing with urgent visa problems and to announce his name, address and telephone number well in advance of the meeting so that contact can be made quickly if and when participants have special difficulties.

Yours faithfully,

L. L. M. VAN DEENEN

University of Utrecht

H. R. V. ARNSTEIN

King's College, London

SIR,—In continuation and support of S. Peller's letter (December 13) I would like to point out that I was, obviously on the same grounds, subjected to an identical procedure by the diplomatic authorities of the host land of the Extraordinary General Assembly of the International Astronomical Union at Torun in September 1973—in spite of possessing an official invitation.

Although I was far from active politically or in any other way that could interfere with the interests of the host country, and despite rigorous adherence to the instructions printed on the invitation, I was refused an entry visa and thus prevented from attending the assembly. I wish to thank all colleagues who tried—in vain—to change the decision at the very last moment.

Yours faithfully,

ANDREI R. SERBAN

Heidelberg

IQ Statistics

SIR,—A recent editorial (September 27) has drawn attention to the unfortunate social consequences of the recent con-

troversy about the possibility of genetic contributions to the observed differences between the average IQs of different races. It does not seem to have been noticed that many of the contributors to the debate, on whatever side, have quite misunderstood the logic of the relevant statistical methods.

If, as a first approximation, we assume that the observed IQ of an individual is the sum of a genetic component, G , and an environmental component E , so that $I = G + E$, it has become customary to define a 'coefficient of heritability', h^2 , as the ratio of the variance of G to that of I . In doing this the tacit assumption is made that G and E are uncorrelated in the population so that the variance of I is the sum of the variances of G and E . This is usually the case for quantitative characters in animals and plants. For human IQ, however, there is no doubt whatever that G and E are correlated, environment (both physical and psychological) being strongly associated with intelligence. Thus there is no point in attempting to estimate h^2 since it cannot be defined, let alone estimated, and attempts to estimate in are a misapplication of the analysis of variance.

It is of course, true that it might be possible in theory (but very difficult in practice) to divide the variance of I into three terms, the variances of G and E , and their covariance, and even to go further and split the variance of G into linear, dominance and epistatic components.

Even if this could be done, however it is clear that such components of variance within a population throw hardly any light on the differences between populations. This is easy to illustrate on animal and plant populations where h^2 within populations, when it can be defined, has no relationship to population differences.

At present there exist no methods of estimating differences in mean levels of G , and there is no evidence of any kind which suggests that negro intelligence is, on the average, less than, equal to or greater than that of white people. These three hypotheses are not intrinsically unpalatable but those who think they can be tested empirically have been inadequately instructed in the analysis of variance by their statistical colleagues.

Yours faithfully,

P. A. P. MORAN

The Australian National University

University competition

SIR,—J. B. S. Price's lament about unfair competition from university-inspired science-based companies cannot be allowed to go unanswered.

One has sympathy for a company like Mr Price's which attempts to make profit from difficult technology—too often the development costs are unlikely to be justified by the potential market. Science often requires such development to advance a subject, however, and without it no market could possibly develop. It is just such devices that are likely to be spawned by university staff. The important question for the scientific instrument industry in the UK is: How do we get such schemes financed? Certainly not from companies like Mr Price's, in 1975. And only partially from the NRDC—50% on very hard terms.

I am surprised he fears university competition on hardware. No university can operate such activities except on a very small scale and has no sales organisation. If the scale is small, it is unlikely to be profitable for such companies as Grubb Parsons in any event and perhaps should be tackled by a smaller company.

He has less to fear than he thinks; such operations are rarely very professional and most buyers won't trust universities to come up with the goods—again keeping the scale small. There are, of course, university entrepreneurs who expand their activities rapidly, becoming fully commercial and professional. They then, however, face all the problems of production facilities, cash-flow and overheads that Mr Price does. Their sole advantage is their enterprise and enthusiasm.

I write because a negative attitude to this problem will only lead to feather-bedding; with enough of that, the competitiveness of British scientific industry will continue to diminish to the disadvantage of our balance of payments.

Whatever is said, we must find an effective way to turn the country's considerable investment in university research into industrial progress.

Yours faithfully,

S. D. SMITH

Heriot-Watt University

Taboo research?

SIR,—Some 20 years ago the first infectious virus was reconstituted in the laboratory from component chemicals, and some religious circles, Catholic as well as fundamentalist, were wondering whether to disapprove such research activities on the grounds that they were approaching too close to God's territory. They wisely and fortunately remained silent.

This week an international group of scientists and science administrators

met at a resort on the coast of California to discuss the merits, or rather dangers, of a much more advanced research on similar lines which is going on all over the world. I hope that this group will show as much wisdom as the churches did earlier. Taboos to block, or guidelines to channel man's curiosity have never succeeded in the long run, and usually they appeared ridiculous a few decades later. They are as old as the caveman's wife telling her mate not to bring that burning piece of wood from the tree hit by lightning into the cave, and not to pick up that club-shaped branch when attacked by a bear. Yes, there was danger in these activities—man could and did hurt himself as a consequence of each of these acts. The end effect of these activities, however, is that *homo sapiens* has greatly multiplied and that ever more members of the species live longer, more healthily and more comfortably than before.

Another result is that people are now able to arrange world-wide meetings, with busy scientists travelling through the air, very rapidly and rather safely, to discuss further steps in unknown directions, and their dangers, and guidelines to hamper these steps. Man also developed remarkable means of communication so that the original fears and misgivings of the caveman's mate ('caveperson', so as not to prejudge who brought the fire in) are now brought to everybody's attention and the weak, timid, conservative (not to say conservationist) members of the species may through their numbers be able to exert real pressure on the Promethean creative curious individuals, to make them cease and desist. The human species as a whole has, however, survived, and benefited, from the discovery and utilisation of fire, the wheel, powder, the steam engine, vaccination, electricity, dynamite, fluoridation and atomic energy. It will surely hardly notice that some scientists are suffering from extrapolatosis and super-scrupulosis. Thus the danger of the present meeting to future research is surely small, if any, and of short duration. The fact that this is a court of scientists sitting in judgment on scientific research makes it more disturbing and regrettable, however, in that it shows to what extent the anti-intellectual disease of doubting the value of progress and advance of knowledge has spread to the ranks of the presumed Prometheans.

H. FRAENKEL-CONRAT

University of California

Naming names

SIR,—I would like to align myself with those anthropologists, such as Robin A. Drews, who have a proprietary attitude

toward the proper spelling of Peking man (Peking, please, not Pekin). My own proprietary attitude extends to the Peking duck (*Anas platyrhynchos*). Although it is probably too late to do anything about Pekin, Illinois, USA, I do hope we can rescue the "... big, white duck known as a Pekin variety" from nomenclatural ignominy.

First of all, there is no Pekin, China. The name Peking comes from two Chinese characters, the first of which (pei) means northern and the second of which (ching or king) means capital. Although some anthropologists suspect 'Americanism at work' in the base misspelling of their favourite fossil remains and my favourite domestic waterfowl, I have developed a different hypothesis in the course of years of grappling with this persistent problem in orthography.

The Peking duck originated in China and was first noted in the USA around 1870, but I can't determine from whence it came. I had always assumed Europe. While reading some marginalia in Jean Delacour's engaging volumes on waterfowl systematics, I observed that he claimed to have visited Pékin, China in the course of his studies. I was a little dismayed that such an obviously literate and well travelled scientist could be found among the ranks of orthographic incompetents until I learned that in the French language Peking (whether city, duck, or man) is Pékin. Thus, I arrived at an essentially francophilic explanation of the entire business; to wit, the forlornly foreshortened Pekin represents the uncritical (and incomplete) adoption of the French word for Peking. *Voilà!*

GILBERT GOTTLIEB

Raleigh, North Carolina 27611

SIR,—The term 'exobiology' excludes life on Earth. It is, of course, impossible at present to study exobiology without reference to life on Earth. Even if several extraterrestrial life forms were discovered tomorrow, nobody would study them without comparing them with terrestrial life. Thus there is a need for a word which includes all life forms in the universe.

The term cosmochemistry is already well established and has been defined as "the branch of science which treats the chemical composition of the universe and its origin and evaluation". It would thus seem to be appropriate to use the term cosmobiology for the study of life in the Universe. I believe that such matters of terminology are not trivial, for the manner in which a science is divided into branches has a significant effect on the development of that science.

Yours faithfully,

A. A. COTTEY

University of East Anglia

news and views

Chloroplast protein synthesis

from Harry Smith

READERS of the more distinguished biochemistry and molecular biology journals could be forgiven for concluding that plants do not have genes, nucleic acids, proteins, enzymes and intriguing regulatory processes such as are possessed by respectable organisms like *E. coli* and HeLa. The omissions are doubtless due to the small amount of work in progress in these areas of plant science, which is, in turn, largely caused by lack of interest in, and knowledge of, plants on the part of traditionally trained biochemists. It is, therefore, refreshing to see a group tackling one of the central problems of plant molecular biology and in the process producing a concept of interorganelle regulation which may have important implications for other organisms.

R. J. Ellis and his colleagues at the University of Warwick have for several years now been studying the complex problem of the synthesis of Fraction 1 protein in leaves. This protein, which may represent up to 50% of the total soluble leaf protein, is only found in its intact state within the chloroplasts, where it carries the catalytic function of ribulose-1,5-diphosphate carboxylase, the enzyme responsible for photosynthetic carbon fixation. The molecule has a molecular weight of about 525,000 and probably consists of eight identical large subunits (molecular weight about 55,000) and eight to ten identical small subunits (molecular weight about 15,000). Genetic experi-

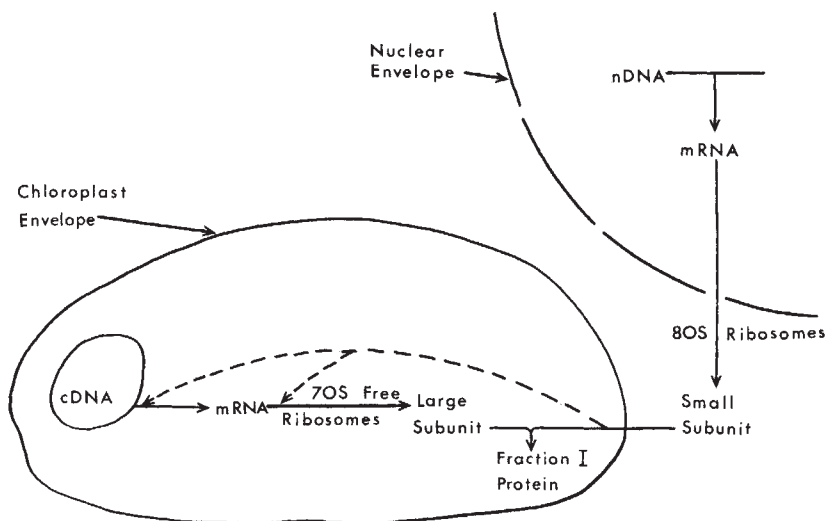
ments have shown that the functional gene for the large subunit is present in the chloroplast genome, whereas that for the small subunit is in the nuclear genome. Moreover, earlier inhibitor experiments indicated that the large subunit is synthesised only on chloroplast ribosomes *in vivo*, and the small subunit on cytoplasmic ribosomes. There is thus a fascinating problem of interorganelle communication and integration.

In 1973 Blair and Ellis (*Biochim. Biophys. Acta*, **319**, 223–234) showed that intact, isolated pea chloroplasts when driven by light energy, incorporated labelled amino acids into the large subunit of Fraction 1, the only soluble polypeptide to be synthesised. Its identity was established by comparing a tryptic map of the labelled peptides with that from authentic Fraction 1 large subunit. Subsequently, Eaglesham and Ellis (*Biochim. Biophys. Acta*, **335**, 396–407; 1974) showed that isolated chloroplasts in the light incorporate label into six separate membrane-bound products which were considered to be membrane proteins. Other workers have obtained similar results using spinach chloroplasts, but in this case upwards of four soluble proteins, and nine membrane proteins, became labelled (Bottomley, Spencer and Whitfield, *Archs Biochem. Biophys.*, **164**, 106–117; 1974). The concept has thus developed that at least Fraction 1 protein large subunit and a

number of chloroplast membrane proteins are synthesised on chloroplast ribosomes. The location of the genes for the membrane proteins is not yet known.

Hartley, Wheeler and Ellis have now demonstrated (*J. molec. Biol.*, **91**, 67–77; 1975) the existence of the messenger RNA for the large subunit by the only real criterion—its *in vitro* translation into a recognisable polypeptide. Using a cell-free *E. coli* preparation as a heterologous protein-synthesising system, they showed that the Fraction 1 large subunit is synthesised when total spinach chloroplast RNA is presented as the message. Two major labelled products were found, both in the cell-free heterologous system, and as a result of protein synthesis by intact light-driven chloroplasts. One of these, with a molecular weight on SDS gels of 52,000, had a closely similar chymotryptic map to that of authentic Fraction 1 large subunit. The evidence for the identity of the labelled product with Fraction 1 large subunit seems most convincing.

Since the large subunit is coded for in the chloroplast genome, its messenger RNA is present in chloroplast RNA, and it is synthesised on chloroplast ribosomes, some control of synthesis must be exerted such that the relative proportions of large and small subunits are coordinated. In the January issue of *Phytochemistry* (**14**, 89–93; 1975) Ellis puts forward a very intriguing hypothesis for the mechanism of this integration. He shows that MDMP (2-(4-methyl-2,6-dinitroanilino)-N-methylpropionamide), a highly specific inhibitor of initiation on 80S ribosomes, inhibits the synthesis of both the large and the small subunit when applied to intact pea leaves. MDMP does not inhibit protein synthesis in chloroplasts. On the basis of this evidence, a model is proposed in which the small subunit is thought of as a positive factor required for the initiation of either the transcription of the messenger RNA for the large subunit, or for its translocation (see figure). On this model, the synthesis of the large subunit by isolated chloroplasts represents run-off of preformed polyribosomes, which accounts for the observed rapid falling off in the rate of protein synthesis. This elegant and painstaking work convincingly demonstrates that plant molecular biology is not dead, but is merely awaiting the attention of competent and dedicated biochemists.



Cause and effects of global cooling

by John Gribbin

A RECENT flurry of papers has provided further evidence for the belief that the Earth is cooling. There now seems little doubt that changes over the past few years are more than a minor statistical fluctuation.

Last year, Kukla and Kukla (*Science*, **183**, 709; 1974) reported satellite observations of the sudden growth of ice cover in the northern hemisphere in the winter of 1971–72, and suggested that the increase was equivalent to one-sixth of the change needed to bring about a full ice age. On page 45 of this issue of *Nature*, Wahl and Bryson compare recent sea surface temperature patterns with those of cooler regimes in the past, and conclude that over the period from 1951 to 1972 there was a decline corresponding “to a return of about one-sixth of the way to a full ice age”. It is tempting to suggest that the change in sea surface temperatures contributed in some way to the spread of ice noted by Kukla and Kukla.

But there is as yet no evidence that further cooling is likely in the immediate future. The observed cooling

corresponds to a re-establishment of the ‘Little Ice Age’ which persisted for several hundred years up to the end of the nineteenth century; it may be that all that has happened since 1950 is that the unusually mild spell of the first part of this century has ended. Over the past millenium, icier conditions than those of today have been the norm. Indeed, ice ages have been common over the past two million years. But on longer time scales still, such cool phases are relatively rare events—and there is now a plausible model to explain why this should have been so.

At present, the land surface of the Earth is concentrated in the northern hemisphere, around the almost land-locked polar sea. This is a situation which can encourage the spread of ice much more than if warm water could easily penetrate to the polar sea, although of course polar ice forms even more easily when the pole is covered by land, as in Antarctica today. It seems that over the past couple of million years this distribution of the continents has resulted in a fairly delicate

balance between ice ages and interglacial regimes. If ice cover is established, its high albedo is enough to maintain glacial conditions for a long time; but once the ice has gone a definite trigger (such as volcanic activity or changes in the Earth’s orbit; see page 20) is needed to re-establish it.

Calculations presented by Sellers and Meadows (on page 44 of this issue) support the commonly held view that the present-day distribution of northern continents may be the root cause of the wave of ice ages in the past two million years. Neglecting other effects, if the continents were gathered in a belt around the equator, Sellers and Meadows find that the difference in albedo from conditions like those of the present would raise the global mean surface temperature by 12 °C.

Much more work of this kind needs to be done before plate tectonics can provide a full insight into the long-term changes in patterns of glaciation, but at least the link between continental drift and ice ages is now beginning to be put on a quantitative basis.

Eukaryotic operon

from P. J. Ford

GENETIC regulatory systems in prokaryotes are quite well understood compared with those in eukaryotes. Regulator genes code for regulator substances (usually proteins in prokaryotes) which function by interacting with regulator sites (operators) in the DNA adjacent to the structural genes under control and with small molecules (either inducers which are usually substrates or repressors which are usually end products) which alter their affinity for the regulator sites. A simple model supposes that regulator substances exist in two forms, one capable and one incapable of binding to the regulator site. Inducers and repressors shift the equilibrium between these two forms.

Four types of gene control circuit are theoretically possible using these components. Negative control exists when the binding of regulator protein (repressor) at the regulator site inhibits gene transcription. In this case gene activity may be restored (induced) by inactivation of the repressor in the presence of inducer or eliminated (repressed) by activation of the repressor protein in the presence of co-repressor substance which is essential for repressor activity. Positive control exists when binding of regulator protein (inducer protein) at the regulator site is

essential for gene transcription. Inducible positive control is found when the presence of an effector substance (co-inducer), as well as inducer protein, is required for gene activity and repressible positive control occurs when the co-repressor inactivates the inducer protein, eliminating gene activity.

Regulator gene mutants in negative control circuits must be recessive if structural gene function occurs in the absence of inducer (constitutive) or in the presence of co-repressor (de-repressed); or dominant if there is no expression in the presence of inducer (uninducible) or in the absence of repressor (super-repressed). In positive control circuits the dominance and recessive relationships are reversed. Mutations in regulator sites are always dominant when linked (*cis* configuration) to structural genes and recessive when unlinked (*trans* configuration). Mutations in regulator sites of negative control circuits are constitutive or de-repressed and of positive control circuits are uninducible or super-repressed.

These four theoretically possible types of simple control circuit are not equally used. Most control circuits in prokaryotes are under negative control and either inducible, like the lactose

operon, or repressible, like the tryptophan operon. An inducible positive control system is the cyclic AMP system which controls several inducible operons (galactose for instance) and such operons are under dual control. In this system cyclic AMP, which is neither a substrate for, nor a product of the enzyme pathway, activates a binding protein which is required for transcription of the operon. A further complication may arise when a regulator protein acts both in a negative and a positive way such as is found in the control of the arabinose operon. In this circuit the regulator protein acts as a repressor in the absence of arabinose but in its presence binds to an initiator site, allowing gene activity.

A second type of control site (called promoter or polymerase binding site) which may be completely separate from or partially overlap the operator site has been found in many prokaryotic control circuits. Promoter sites are usually located distal to negative and proximal to positive control operators. Theoretically, positive control may be exercised by interaction of control proteins (sigma factors) with the polymerase core enzyme altering its specificity towards promoter sequences. Mutations in promoter sequences will



US Department of the Interior

PART of North America's largest herd of caribou which migrates yearly between forest and tundra in north-western Alaska. They regularly cross the basin of the Noatak river, one of the proposed National Wildlife Refuges which will be created as a result of the Alaska Native Claims Settlement Act of 1971.

The Noatak region lies in a transi-

tion zone between northern boreal forest and arctic tundra. With 500 species of vascular plants the flora of the proposed 7.5 million acre refuge is as great as that of the whole of Greenland or Iceland. The Department of the Interior intends to establish a 20-year moratorium on any developmental activities in the region to allow a comprehensive analysis of

this intact slice of arctic ecosystem to be conducted. At a time when immense pressures are being applied to arctic ecosystems, in the form of roads, pipeline corridors and oil and gas extraction fields, the decision is as wise as it is opportune. What is learned from the analyses will benefit not only the future of the Noatak region but perhaps the future of all arctic lands.

be *cis*-dominant and *trans*-recessive and may be either 'up' (increasing transcription) or 'down' (decreasing transcription) in phenotype.

Clustering under common genetic control of the structural genes for enzymes of the same metabolic pathway is seen in prokaryotes but not eukaryotes, and represents an apparent distinction between these major phylogenetic groups. Taken together with their greater genomic complexity, the presence of large amounts of redundant DNA sequence and their intricate patterns of development and differentiation one might expect the gene control processes of eukaryotes to involve something more than those seen in prokaryotes. But the genetic analysis of gene control in eukaryotes has hardly begun and it is too early to say whether this expectation will be fulfilled. Two articles (Arst and MacDonald; page 26; Arst and Scazzocchio, page 31) in this issue of *Nature* identify regulator sites involved in the control of two enzyme systems in the ascomycete fungus *Aspergillus nidulans*.

Proline catabolism in *Aspergillus* involves three enzyme activities: a permease, an oxidase and a dehydrogenase, which are under three distinct forms of control—induction by proline, nitrogen metabolite repression, and carbon catabolite repression. A probable structural gene for the permease (*prnB*) is tightly linked to a probable structural gene for a component common to both the oxidase and dehydrogenase (*prnA*). *prnA*⁻ mutants lack oxidase and dehydrogenase and *prnB*⁻ mutants lack the permease. All three activities are inducible, permease and oxidase are carbon catabolite repressible (dehydrogenase was not investigated), but only oxidase and dehydrogenase are nitrogen

metabolite repressible—the permease is not. The two repression systems seem to act independently of each other. A gene *areA* is probably a regulator gene producing a positive control element which allows the synthesis of a large number of enzymes and permeases involved in nitrogen metabolism. One class of mutants (*areA*^r) leads to inability to use a variety of nitrogen sources including proline and presumably lacks a functional *areA* product. Second site mutations, called *prn*^d, restore the ability of *areA*^r mutants to utilise proline as a nitrogen source. *prn*^d mutations are *cis*-dominant, *trans*-recessive mapping between but not suppressing or allelic to *prnA*⁻ or *prnB*⁻ mutations, which are themselves tightly linked. *prn*^d mutations do not significantly affect the induced levels of enzymes and are probably best interpreted as carbon catabolite derepressed since uninduced levels of the products of both *prnB* (which is not ammonium repressible) and *prnA* are higher in *prn*^d mutants than in wild type in the presence of partial glucose repression.

The model favoured by Arst and MacDonald to explain their results of a regulator site (*prn*^d) located between two structural genes (*prnA* and *prnB*) is reminiscent of the divergent arginine operon in *E. coli*. They suggest that *prn*^d may be the binding site for a negative control element produced by a gene, such as *creA*, identified by mutations which are commonly recessive and lead to carbon catabolite derepression.

In a second paper Arst and Scazzocchio describe the isolation of a second site revertant (*uap*-100) of a mutant *are*-102 which has reduced levels of, among other enzymes, uric acid/xanthine permease. *uap*-100 is tightly

linked to the probable structural gene (*uapA*) for uric acid permease. It is *cis*-dominant, *trans*-recessive and after maximal induction has a permease activity 2.5 times that of fully induced wild type. They interpret these results as identifying a regulator site with promoter-like properties.

Although the description of these two control systems is at an early stage it seems likely that Arst and his colleagues have identified an operon-type structure involved in proline catabolism and a promoter-like regulator site adjacent to the putative structural gene for uric acid permease in the eukaryote *Aspergillus nidulans*. The recent results of Hynes (*Nature*, **253**, 211; 1975) suggest that a similar regulatory mechanism affects the structural gene for acetamidase in *Aspergillus nidulans*.

Are mantle plumes jets or blobs?

from Peter J. Smith

IN a series of reports appearing over the past two years or so, Schilling and his colleagues (see, for example, Schilling, *Nature*, **242**, 565; 1973 and Hart *et al.*, *Nature phys. Sci.*, **246**, 104; 1973) have presented geochemical evidence which they claim is consistent with, and thus appears to support, the existence of a rising mantle plume beneath Iceland. In particular, they have interpreted the chemistry of basalt lavas from Iceland and surrounding areas in terms of two distinct magma sources—the conventional asthenosphere and a plume of hot primordial material derived from a much greater depth in the mantle. O'Hara (*Nature*, **243**, 507;

1973), on the other hand, criticised this view on the grounds that the data could as easily be explained in more conventional terms. He pointed out that the geochemical variations radiating from Iceland could be caused not by the mixing of two primary magmas but by differential fractional crystallisation of material from a single source; and he now (in *Nature*, **253**, 708; 1975) reiterates, clarifies and expands the arguments in support of his case.

In his original article O'Hara seemed to imply that a choice would have to be made between two mutually exclusive models. He now suggests that fractional crystallisation and a mantle plume might be compatible under certain circumstances but that, if they are, Schilling's particular interpretation involving relatively undepleted plume material must certainly be ruled out on grounds of density alone. Schilling, however, continues to stick to his own model involving a plume "made of slightly lighter and more primordial material"; and he and Noe-Nygaard (*Earth planet. Sci. Lett.*, **24**, 1; 1974) have now extended the investigation to cover variation in the behaviour of the Icelandic plume with time.

In the light of Schilling's view that the plume comprises material chemically and isotopically distinct from that of the asthenosphere (which is penetrated by the plume from below), Schilling and Noe-Nygaard envisage three different types of plume-ridge relationship. Their 'normal ridge segments' (NRS) remote from any plume have normal ridge elevation, well developed and symmetrical magnetic anomalies and typical oceanic crustal thickness and structure. The material rising to form new lithosphere (in response to spreading rather than as a cause of it) is derived from the asthenosphere, and the resulting basalts are tholeiitic, depleted in large ionic lithophile elements and low in radiogenic isotopes, and have $(La/Sm)_{EF}$ ratios <1 .

The 'plume ridge segments' (PRS) directly over plumes have higher elevation, branching aseismic ridges or island chains, crustal thickness and structure intermediate between the typically oceanic and typically continental, and higher geothermal gradients. Here plume material is forced into the gap left by diverging plates, usually in quantities sufficient to more than fill the gap and thus allow the accumulation of plateau basalts and the formation of anomalously thick oceanic crust. In this situation new lithosphere over a PRS will be derived entirely from the plume, will have $(La/Sm)_{EF} >1$ and will have radiogenic isotopes characteristic of the particular plume. But when and if the plume flux decreases to below the quantity required by the diverging plates, asthenospheric



A hundred years ago

THE destruction of seals in the Arctic seas has been carried on to such an extent that fears are entertained of the annihilation of these animals. The Peterhead sealers and whalers have therefore determined to agree to a "close time," during which it shall be unlawful for any sealing-ship to kill seals, or even to leave port for the fishing-grounds; thus giving the newly-born seals time to develop into a useful size, and enabling even the parent-seals to escape. It is hoped to extend this regulation to other countries engaged in the industry; and the Board of Trade has been in correspondence with various authorities on the subject. The papers in connection with the case have been presented to Parliament, and will shortly be printed, when the decision of the Government will probably be made known.

from *Nature*, **11**, 373; March 11, 1875

material will mix with the plume material leading to hybrid lavas with intermediate $(La/Sm)_{EF}$ values and radiogenic imprinting. Finally, there are also 'transitional ridge segments' (TRS) between NRS and PRS, but for the time being these are ignored because of their complexity.

With this model (NRS and PRS only) in mind, Schilling and Noe-Nygaard have investigated the rare earth element (REE) chemistry of the 3,000 m thick lava sequence in the Faroe Islands. This pile of plateau lavas contains two clear marker horizons which define a series of aphyric quartz tholeiites (lower), a series of porphyritic quartz tholeiites (middles) and a series of olivine tholeiites (upper). The geochemical study shows that, with the exception of one flow in the middle series, all the flows of the lower and middle series have light REE enrichment with $(La/Sm)_{EF} >1$. By contrast, the flows of the upper series fluctuate rapidly between REE depletion with $(La/Sm)_{EF} <1$ and REE enrichment, ending (at the top) with flows predominantly depleted. Schilling and Noe-Nygaard claim, giving reasons, that this two-component pattern cannot be attributable to differing fractional crystallisation or partial melting. They argue instead that the data support their two-source model. The lower and middle series are plume-derived, with the exception of the anomalous middle series flow which marks the first appearance of asthenospheric material. In the upper series there are rapid

changes between flows mostly plume-derived and flows mostly asthenosphere-derived, with the most recent activity being mainly of the latter type.

But why did the change from plume material to largely asthenospheric material take place? There are two possibilities within the scope of the Schilling-Noe-Nygaard model; either the plume must have decreased in intensity or the spreading rate at the ridge must have increased, thereby allowing the asthenosphere to acquire greater influence. According to Tarling and Gale (*Nature*, **218**, 1043; 1968) the ages of the Faroes basalts lie between 60 and 50 million years, and according to Vogt and Avery (*J. geophys. Res.*, **79**, 363; 1974) spreading in the area was decreasing rapidly at that time. The intensity of the plume must therefore also have decreased rapidly. Before it did, however, spreading rate and the flow REE chemistry were comparable to those relating to Iceland during the past few million years, which suggests that the plume intensities then and now were similar. This, in turn, would imply that the plume intensity went through a minimum some time between 50 and 10 million years ago.

Insofar as so very little is known about the origin of supposed mantle plumes and the conditions of their existence, there is presumably no basis for ruling out such temporal variations in plume intensity. On the other hand, in view of the large inertia of thermal systems of the required size, it may be difficult to understand how a plume envisaged as a continuous jet of material could vary in intensity quite so rapidly as the geochemical properties of the Faroes basalts imply. One way in which the REE data and a continuous plume could be reconciled would be to postulate sudden rift jumping and tapping of asthenospheric material at the plume margin, but there is apparently no observational support for this. Schilling and Noe-Nygaard therefore propose that mantle plumes may not be continuous jets at all but may arise as a series of discrete blobs or diapirs. This makes rapid flux variations in space and time much easier to visualise, especially if the rising blobs were to taper downwards rather as inverted liquid droplets.

Reducing exposure of tissues to contraceptives

from our *Steroid Biochemistry Correspondent*

SINCE the late 1950s when steroids were first used as oral contraceptives many variations in the nature of the steroid

employed, the mode of administration and the dose administered have been tried in order to increase the acceptability of hormonal contraceptives and to eliminate possible side-effects. But the fact that the steroids enter the general circulation means that tissues other than those concerned with the control of fertility are exposed to the biologically active compounds, and often for long periods of time. In order to overcome these disadvantages the possibility of obtaining an antifertility effect by a more direct action of the steroid on tissues of the reproductive tract, with a consequent reduction in the systemic effect, has been investigated.

One interesting development uses the advantageous features of an intra-uterine device and low-dose progesterone contraception (Pharriss *et al.*, *Fertil. Steril.*, **25**, 915-921; 1974). This intra-uterine device is made of an ethylene copolymer in the shape of a T, the stem of which is hollow and contains a suspension of microcrystals of progesterone. The rate of release of progesterone from the device is controlled by the ethylene copolymer membrane. Initial trials suggested that a progesterone release rate of 65 µg per day was sufficient to provide a contraceptive effect. This amount of progesterone is equivalent to only a small proportion of the daily production rate during the luteal phase of the menstrual cycle. The present report gives details of the use of this device by more than 3,000 women for more than 25,000 women-months of treatment. The efficacy and use effectiveness of the device compared favourably with that of other methods of contraception. The pregnancy rate was only 1% compared with a rate of 18% for a similar T-shaped device not containing progesterone. A T-shaped device was used because of its acceptability, easy insertion and removal and low natural expulsion rate.

This method of contraception has other advantages: the active compound is delivered directly to the target tissue and the low release rate of the natural ovarian hormone is unlikely to produce any systemic effect even if any enters the general circulation. The sloughing of the endometrium at menstruation prevents an accumulation of the steroid in this tissue. In investigations with monkeys using devices containing isotopically labelled progesterone it was shown that some radioactivity did enter the circulation but since the uterus is known to metabolise progesterone it seems that this radioactivity is more probably associated with biologically inactive metabolites than with progesterone itself. The total plasma radioactivity remained constant over a one-year period showing that a relatively constant release rate of pro-

gesterone from the device was obtained. As with other intrauterine devices this system has the advantage that it does not require any active involvement by women using it.

An alternative approach to the problem of reducing the continuous exposure of the tissues to the contraceptive steroid involves the administration of a synthetic progestogen at the middle of the menstrual cycle before endogenous progesterone secretion commences (Sakiz *et al.*, *Contraception*, **10**, 467-474; 1974). In this way the synthetic steroid blocks the progesterone receptors in the uterus and prevents endogenous progesterone from inducing those changes in the uterus which are necessary for implantation to occur. In a trial involving 140 women who completed 1,362 treatment cycles, 50 mg of the progestogen ethynorgestrienone was administered on days 15, 16 and 17 of the menstrual cycle. The method proved to be an acceptable form of contraception for most women and many of the minor side-effects seen with other hormonal contraceptives seemed not to occur. But there was a failure rate of 9.6 per hundred women years which is too high to be acceptable; about half of these pregnancies were considered to be due to method failure. One difficulty of the method is determining the time of ovulation precisely since ideally the progestogen should be administered immediately after ovulation. The approach seems however to be a promising one.

IgE and immunity to schistosomiasis

from F. E. G. Cox

ONE of the characteristics of the immune response to most infections caused by parasitic worms is the production of large amounts of IgE. It has been speculated that this immunoglobulin (which is associated with various allergies but does not seem to have any beneficial role) originally evolved as an integral part of the immune response to helminths. Until recently there has been little real evidence to support this hypothesis and theories of immunity to helminths have relegated IgE to a subsidiary role. All observers agree that the mechanisms by which animals rid themselves of worms and resist further challenge are complex but attempts to tie all the available experimental evidence together in a single coherent story make the mechanisms very difficult to comprehend. It is tempting to use all the known facts and to postulate mechanisms involving cell-bound IgE, serum immunoglobulins, lymphocytes, mye-

loid cells, macrophages, complement and various pharmacologically active substances to account for the immune response.

Much of what we know about helminth immunity is based on experiments with various nematodes, particularly *Nippostrongylus brasiliensis* in the rat. The immune response seems to proceed in three steps: damage by antibody, a cell-mediated response and, finally, myeloid cell degranulation and the production of 5-hydroxytryptamine and histamine which leads directly to the expulsion of the worms. The final stage is the one that involves IgE and thus homocytotropic antibody is probably not directly concerned with damage to the worms but plays a peripheral part in the development of immediate hypersensitivity. Apparent confirmation of this peripheral role comes from the work of Jarrett and Bazin (*Nature*, **251**, 613; 1974) who used the recently available purified rat IgE from myelomas as a reference against which to measure total IgE concentrations in animals infected with *N. brasiliensis*. They concluded that the bulk of the IgE is surplus to the concentrations expected against known antigens and probably results from potentiated responses to miscellaneous unknown antigens to which the animals had previously become sensitised.

In schistosomiasis it now seems that IgE has a central role in the immune response. All investigators agree that the stage in the life cycle most susceptible to the immune response is the schistosomula, the young form that penetrates the skin and migrates to the blood vessels in which the adult develops. Clegg and Smithers (*Int. J. Parasitol.*, **2**, 79; 1972) demonstrated convincingly that the schistosomula of *Schistosoma mansoni* were killed *in vitro* in the presence of IgG and complement components from immune rhesus monkeys and Capron *et al.* (*Int. J. Parasitol.*, **4**, 613; 1974) have found similar cytotoxic activity in humans. Antibody-mediated damage can therefore be regarded as the first step in the immune response to schistosomiasis as in *N. brasiliensis* infections.

Various kinds of cell have been shown to be involved in immunity to schistosomiasis and it is difficult to determine the actual role of each type. Dean, Wistar and Murrell (*Am. J. trop. Med. Hyg.*, **23**, 420; 1974) have found that rat neutrophils damage schistosomula *in vitro* by direct contact and by enzymic activity. IgG is involved in the attachment of the cells to the worms and complement also seems to play a part in this process although its role is not proven. Complement-independent killing of schistosomula by mixed "polymorphonuclear and mononuclear" leukocytes has been achieved

by Butterworth, Sturrock, Houba and Rees (*Nature*, **252**, 503; 1974). These authors found that schistosomula were damaged in the presence of normal leukocytes and sera from immune patients. The nature of the cells and antibodies involved is not disclosed but it is suggested that cytotoxicity is associated with eosinophils.

So far neutrophils and eosinophils have been suggested as effector cells in the immune response but the most recent work in this field also implicates macrophages. Capron, Bazin, Dessaint and Capron (*Nature*, **253**, 474; 1974) found that normal rat macrophages adhered to schistosomula in the presence of immune sera *in vitro*. Absorption of the immune sera with various anti-rat-immunoglobulin sera showed the immunoglobulin involved to be IgE. Further experiments showed that the IgE was specific to the parasite, that macrophage adherence played a part in the killing of schistosomula and that complement was not required for the killing. Killing requires the presence of specific IgE which binds normal macrophages to the surface of the worm and, although it may contribute directly to the death of the worm, the main importance of this binding is that it is instrumental in enhancing the lethal effects of immune IgG. These experiments draw attention to the possibility of IgE-mediated macrophage adherence in other situations and at last provide a central rather than a peripheral role for IgE in immunity to helminth infections. Perhaps we will soon know what IgE really does other than cause asthma and hay fever.

Peptidoglycan and the immune response

from a Correspondent

THE bacterial envelope is comprised of the cytoplasmic membrane and the outer cell wall. The classic division of bacteria on the basis of the Gram stain turns out to correspond exactly to variations in the chemistry and structural organisation of the outer part of the envelope. But in spite of the variations there is a unique component, the peptidoglycan, present in greater or lesser amount in the cell wall of all bacteria. The peptidoglycan matrix is built up from strands of chitin-like polysaccharides but with every alternate sugar residue being N-acetylmuramic acid (the 3-O-D-lactic acid ether of N-acetylglucosamine). The acid residues are substituted by amide linkages with short peptide side chains and many of these chains are joined to link adjacent polysaccharide strands together in a meshwork. In most—per-

haps all—gram negative bacteria and in several gram positive bacilli the peptidoglycan structures differ chemically only in detail although it seems probable that their three dimensional arrangements vary considerably. Outside this range of organisms the chemical differences, particularly in peptide structure, multiply enormously.

All this has been known for several years. It is therefore surprising that so little is known about the effects of the ubiquitous peptidoglycan on mammalian cells. Most people are in contact with bacterial peptidoglycan from very early life. Certainly peptidoglycan is immunogenic and there must be a continuous stimulation of the immune response by bacteria indigenous to the respiratory and gastrointestinal tracts.

And peptidoglycan is of course a component of complete Freund's adjuvant—the suspension of tubercle bacilli which, injected as a water-in-air emulsion with antigen, greatly enhances the immune response to many antigens. Does peptidoglycan play any part in adjuvanticity? It now seems clear that it does.

Purified peptidoglycan enhances antibody production and induces delayed-type hypersensitivity at rather lower concentrations than those of the whole bacilli used in Freund's adjuvant. There is a fascinating argument going on between two French laboratories about the minimum structural features of peptidoglycan necessary for the adjuvant effect. Lederer and his colleagues Adam, Ciorboru Ellouz and Petit (*Biochem. biophys. Res. Commun.*, **59**, 1317–1325; 1974) have taken degradation fragments of peptidoglycan from *Mycobacterium smegmatis* or *Escherichia coli* and found that a monomeric fragment N-acetylglucosaminyl (β 1, 4) N-acetylmuramyl-L-alanyl-D-isoglutaminyl-meso-diaminopimelyl-D-alanine can replace whole killed mycobacteria or undegraded peptidoglycan. Furthermore the terminal N-acetylglucosaminyl and D-alanyl units can be removed without loss of adjuvanticity. A synthetic compound, N-acetylmuramyl-L-alanyl-D-isoglutamine turns out to be at least as active as the monomeric unit but a synthetic disaccharide-L-alanyl derivative is inactive. So are the hexosamine free di- to tetra peptides. Lederer concludes that the glycopeptide linkage and D-isoglutaminyl unit are indispensable for an adjuvant effect.

Nauciel and Fleck (*C. r. Acad. Sci. Paris*, **276D**, 3499–3500; 1973; *Nature*, **250**, 517–518; 1974; *Eur. J. Immun.*, **4**, 352–356; 1974) disagree on the first point but support the importance of D-isoglutamine. They find that the peptide subunit alone possesses the same adjuvant activity as intact peptidoglycan at least in delayed-type hypersensitivity

reactions to azobenzenearsonate-N-acetyl-L-tyrosine.

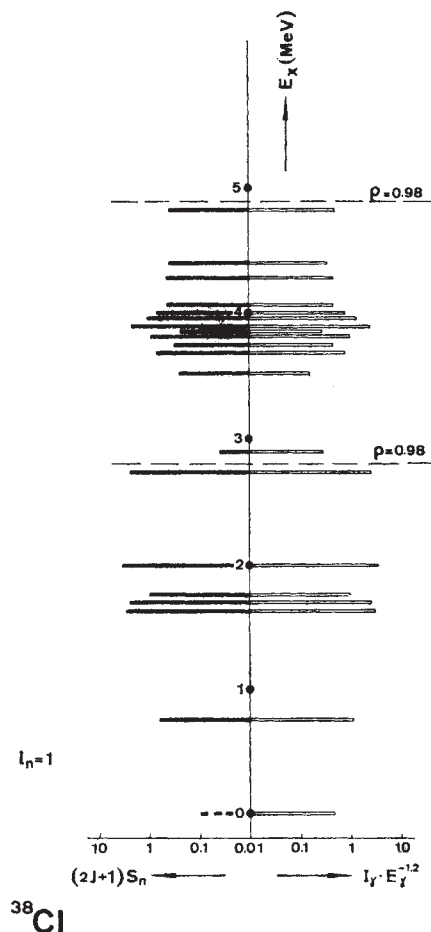
In a search for other points in the immune response at which peptidoglycan might function, the inhibitory effect of this substance on macrophage migration (Heymer *et al.*, *J. Immun.*, **116**, 1743–1754; 1973) is relevant. This effect seems to be distinct from migration inhibition of macrophages mediated immunologically by factors secreted by activated lymphocytes. A direct action of peptidoglycan on the macrophage surface is possible but little is known as yet about the structural features responsible for this interesting effect.

Finally, stimulation of graft rejection—for example of tumour cells—by *Mycobacterium tuberculosis* BCG has been studied by Zbar *et al.*, (*J. natn. Cancer Inst.*, **48**, 831–835; 1972), using isolated mycobacterial cell walls. The effect is complex and, at least in the systems in which virally induced tumours were studied, stimulation of host interferon production by the mycobacterial adjuvant is possible. But this presumably is not involved in host rejection of chemically induced tumours. Could a lectin-like activity of antibodies directed against the polysaccharide part of peptidoglycan be involved in host reactions against tumour cells? Several lectins that agglutinate tumour cells (for example, wheat germ agglutinin: Allen and Neuberger, *Biochem. J.*, **135**, 307–314; 1973) interact with an N-acetylchitobiose unit that is found commonly in mammalian glycoproteins, and also bind bacterial peptidoglycan containing an analogous repeating disaccharide unit. In some interesting experiments Shier (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 2078–2082; 1971) prepared antisera against a synthetic antigen containing N-acetylchitobiose hapten groups. His finding that these antibodies show some antitumour effects therefore raises the interesting question whether antibodies of similar specificity raised in response to peptidoglycan mimic this reactivity.

Single-particle states of nuclei

from P. E. Hodgson

MUCH of our knowledge of atomic nuclei has been obtained by studies of reactions that remove one nucleon from the nucleus or add one nucleon to it. Analysis of the cross sections of these interactions gives the quantum numbers and the occupation probabilities of the single-particle states of the simple shell model. This is a very basic feature of nuclei, and it is related to many other nuclear properties such



³⁸Cl

Fig. 1 Comparison between the stripping strengths $(2J+1)S_n$ for the reaction $^{37}\text{Cl}(d,p)^{38}\text{Cl}$ to many states of ^{38}Cl (solid lines) and the reduced transition probabilities after thermal neutron capture $^{37}\text{Cl}(n,\gamma)^{38}\text{Cl}$ (open lines). Only the transitions with orbital angular momentum transfer $L_N=1$ are included. The (n,γ) partial radiation widths were normalised so that their largest value equals the corresponding $(J+1)S_n$ values. The correlation coefficients are given for levels up to 2.8 MeV and for all levels.

as their charge and matter distributions, electromagnetic transition rates and quadrupole moments.

The theories used to analyse the experimental data, in particular the distorted wave theory, involve certain approximations and are subject to some assumptions that together limit the accuracy of the final results. The formalism contains some purely mathematical approximations, and physically there are for example uncertainties connected with the possible contribution of compound nucleus and two-step reaction processes. Some improvement in the accuracy of these analyses has recently come from the use of the j -dependent sum rules (*Nature*, **249**, 695; 1974). More recently Clement in a contribution to a conference on nuclear structure and spectroscopy held last year in Amsterdam has drawn attention to the importance of comparisons between the (d,p) stripping and the neutron capture data.

The (d,p) deuteron stripping reaction and the (n,γ) neutron capture reaction both deposit a neutron in one of the unoccupied states of the final nucleus, and so both should give the same result for the structure of a particular nucleus. The important quantities are the spectroscopic factors that measure the single-particle strengths of the states in the final nucleus and the corresponding transition probabilities for neutron capture. Comparison of the numbers obtained for the same nucleus by the (d,p) and (n,γ) reactions is thus a good test of the reliability of both analyses.

Such a comparison was made for ^{38}Cl by Spits and Akkermans (*Nuclear Physics*, **A215**, 260; 1973). They measured the cross section for the $^{37}\text{Cl}(n,\gamma)^{38}\text{Cl}$ neutron capture reaction to many states of ^{38}Cl and compared the transition probabilities they obtained from their data with the spectroscopic factors found from the corresponding $^{37}\text{Cl}(d,p)^{38}\text{Cl}$ deuteron stripping reaction by Engelbertink and Olness (*Phys. Rev.*, **C5**, 431; 1972). The results are compared in Fig. 1 and show a remarkably high degree of correlation between the two sets of numbers. Indeed if the data are analysed statistically the correlation coefficient between the two sets is as high as 98%.

Similarly high correlations have now been obtained for a whole series of nuclei, a few cases of low correlation being readily explicable as due to the presence of nearby resonances. The result for ^{38}Cl is thus not an isolated example, but is quite typical.

These astonishingly high correlations between spectroscopic factors obtained by quite different reactions validate at a stroke essentially all the significant approximations and assumptions in the theories of deuteron stripping and neutron capture (Kopecký, Spits, and Lane, *Phys. Lett.*, **49B**, 323; 1974). The reaction mechanisms are so different that it is inconceivable that any perturbing factor in the analysis should have the same effect in the two reactions. The excitation energies of the compound nuclei in the two cases differ widely, so the compound nucleus cross sections must be different and since they do not affect the results they must both be small. Similarly for the two-step contributions (*Nature*, **247**, 179; 1974) which must be quite different in the two types of reaction. All perturbing factors, if important, could only reduce the correlation coefficients and since they are so high the analysis provides strong evidence that both sets of analysis are rather more accurate than has been generally believed. This very satisfactory result should be a welcome stimulus to further analyses of single particle states in nuclei.

New method of determining nuclear quadrupole moment

from P. E. Hodgson

THE recent theoretical work of Clement (*Nuclear Phys.*, **A213**, 492; 1973) on the nuclear shell model has made it possible to express the nuclear quadrupole moment in terms of single particle quadrupole integrals and the spectroscopic factors extracted from single proton transfer reactions. In some cases all the required data are available and the resulting values of the nuclear quadrupole moment are in excellent accord with those determined by atomic methods.

In a previous note (*Nature*, **249**, 695; 1974) the work of Clement and Perez on the j -dependent sum rules was described. This shows how the spectroscopic factors for stripping and pickup reactions on the same nucleus can be related to each other, thus providing multiple checks of the values obtained for the spectroscopic factors and confirming the validity of the distorted wave theory used to obtain them. If all the necessary data are available, the spectroscopic factors can be obtained with an accuracy of better than 10%.

The single-particle quadrupole integrals are simply overlap integrals of two single-particle wavefunctions with the quadrupole operator. These may be evaluated very easily using harmonic oscillator wavefunctions, but the results are rather inaccurate, especially for valence nucleons. Much better results can be obtained with single-particle wavefunctions evaluated as the eigenfunctions of a Saxon-Woods potential with parameters adjusted to give the correct binding energies; these can now be obtained easily with an electronic computer.

Thus for nuclei for which a complete set of data are available it is possible to use the formalism of Clement to calculate the quadrupole moment.

Clement and Perez (*J. Phys.*, **A7**, 193; 1974) have now done this for ^{37}Cl , and find $Q = -0.057 \pm 0.005$ barn which compares very well with the atomic value $Q = -0.062$ barn, showing the reliability of the method. As more spectroscopic factors become available this should become a powerful method of determining nuclear quadrupole moments, which will be especially useful in cases where the atomic value is not easily obtained.

Diode molecules

from our *Molecular Physics Correspondent*

NOT many years ago a distinguished theoretical chemist deliberately left the field, explaining that an important factor in his decision to work elsewhere was the strong possibility, as he conceived it, that no further, totally new and astonishing phenomenon might ever again be discovered at the purely molecular level. Premature as this may have been at the time, and counter-indicated as it may seem by subsequent events—for example in the area of laser photochemistry—it remains broadly true that, in spite of remarkable advances in instrumentation and the mastering of known effects in ingenious ways, the incidence of altogether new ones in the molecular world could be said to have lagged behind the steady stream of named, prizewinning, conference-engendering effects in other subjects. Having said which, one may as reasonably remark that refuge from pessimism can be sought in the ill-defined yet compelling suspicion that what does remain to emerge from the fastnesses of undiscovered molecular behaviour may prove to be very spectacular indeed.

A sound conjecture—because in a way a truism—is that radically new effects are likely to involve some form of electromagnetic, probably time-dependent phenomenon. Stimulated emission fitted this description; the widely publicised speculations about molecular superconductivity are a long shot in the same direction where the merest vestige of an effect would be altogether sensational.

Closer to Earth, yet of undoubted promise, is the recent speculation by Arieh Aviram and Mark Ratner (*Chem. Phys. Lett.*, **29**, 277; 1974) that it should be possible to design molecular rectifiers by careful patching together of suitable donor and acceptor subsystems. The key to this idea is that, by bridging an acceptor unit, perhaps a quinone-like ring system, by a σ -bonded hydrocarbon bridge to an electrophobic unit, perhaps containing methoxy-groups, the necessary current-voltage asymmetry could be achieved. Actually more exotic candidates for donor and acceptor are available and might be tailor-made for the task, with the passive bridge playing an essential part both in providing rigidity and keeping donor and acceptor at arm's length—just out of range of direct level-interactions, though not of tunnelling transfer.

No doubt these bare ideas will need considerable testing and elaboration before it can be claimed that rectifier action is a practical possibility. But, showing admirable willingness to back their level diagrams with some real

mathematics, Aviram and Ratner go on to present a careful perturbational calculation of the current-voltage characteristics of a plausible rectifier structure (actually a simple hemiquinone). This is semi-quantitative quantum mechanics at its best, using relatively crude approximations to warrant an idea and point to further refinements. When the necessary matrix-elements are computed, with reasonable assumptions of ionisation energy and electron affinity, the I-V characteristic is indeed of the form predicted, with an appreciable asymmetric barrier to conduction.

Needless to emphasise, far too much is left to good fortune for the results to approach a theoretical 'proof' of viable rectifier action. Direct electrode-electron tunnelling could swamp the whole effect; the complex problems of metal-organic junctions and crystal field effects have yet to be built in—each might either enhance or kill off the desired effect. Yet the work described has a convincing air of the *premier pas qui coûte* and we may well hear a lot more of the molecular rectifier, perhaps the absolute ultimate in electronic miniaturisation.

Volcanoes and ice ages

by John Gribbin

ACCORDING to Kennett and Thunell (*Science*, **187**, 497; 1975) "volcanic ash in deep-sea sections indicates very high rates of explosive volcanism during the last two million years". This apparent global increase in Quaternary explosive volcanism flies in the face of some cherished geophysical beliefs—but it offers a new insight into the widespread and frequent glacial activity that has occurred over the same period.

Simple tectonic theory would suggest that volcanic activity is related to local events—collisions between blocks of crust which lead to mountain building. But it now seems from study of 320 deep-sea sections drilled around the world during the Deep Sea Drilling Project that there has been a much higher rate of explosive volcanism from both island arc and 'hot spot' volcanoes during the past 2 million years than the 'normal' level of the past 20 million years. And, as Kennett and Thunell point out, "increased Quaternary volcanism coincides approximately with that episode of the Cenozoic marked by major and rapidly fluctuating climatic change".

Just how these two effects may be related remains to be unravelled, but several interesting possibilities are raised. First, it is well established that

under appropriate conditions the spread of volcanic dust in the atmosphere caused by a marked increase in volcanic activity could trigger the onset of an ice age. The dust simply acts as a veil blocking some of the Sun's heat, and if the resulting cooling lasts for long enough for ice cover to become established it may take more than the removal of the dust veil to melt the ice, because the ice's high albedo will continue to ensure that little solar heat is absorbed.

But studies of shorter stretches of the geological record than the 20 million year sample of the DSDP show that although widespread volcanic activity often precedes the spread of the glaciers it is possible either for glaciation to occur without any increase in volcanic activity, or for there to be an increase in volcanism without immediate climatic repercussions. This encourages speculation that there are one or more additional mechanisms of key importance in triggering ice ages. The Milankovitch model, in which phases of cooling are produced by changes in the orientation of the Earth and by 'stretch' of its orbit around the Sun is perhaps the most attractive of these mechanisms, especially in the form in which Kukla has recently presented it (*Nature*, **253**, 600; 1975). To my mind, the most plausible overall model of ice ages is that when the northern hemisphere is at its coolest phase of the astronomical Milankovitch cycle, conditions will be appropriate for ice cover to develop dramatically if some further effect—such as volcanic activity—provides the final trigger.

But the model can also be turned on its head. The repeated loading and unloading of the Earth's crust caused by the advance and retreat of the glaciers might, in fact, trigger increased volcanic activity. And, if each of these processes occurs there could be a snowball effect by which a small climatic fluctuation becomes a full-scale ice age.

Whatever the truth of the matter, these new discoveries emphasise the impossibility of trying to deal with problems in the earth sciences within rigid compartments. The development of plate tectonic theory has already contributed greatly to the study of past climates by explaining how the present state of the globe, with a nearly land-locked North Polar Ocean, differs from the situation in earlier times when the continents were distributed differently and ice ages were extremely uncommon (see page 14 of this issue of *Nature*). And at a more immediately important level, it seems that a better understanding of volcanism, as well as a better understanding of ocean currents and other aspects of earth science, will be an essential prerequisite to a really satisfactory theory of climatic change.

articles

Tilt and strain monitoring of the 1974 eruption of Mount Etna

G. Wadge, J. A. C. Horsfall & J. L. Brander

Geology and Geophysics Departments, Imperial College, London SW7, UK

Using advanced modern equipment capable of continuous monitoring it has been possible to measure the degree of tilt and strain undergone by Mount Etna during a recent phase of volcanic activity. The results are open to interesting interpretation, and the model suggested here, though hypothetical, is quite plausible geologically.

THE measurement of tilt and of distance have proved valuable techniques in the surveillance of volcanic activity¹. The interpretation of the observed deformation associated with a small eruption in January–March, 1974, gives some insight into the eruptive mechanisms operative on Etna, and also provides some indication of the potential of geodetic techniques for the prediction of activity on that mountain.

The tilt transducer used in this study was an Autonetics biaxial borehole tiltmeter consisting essentially of a circular spirit-level mounted at the bottom of an aluminium alloy tube (Fig. 1). Tilt changes were monitored using a pair of a.c. resistance bridges which gave an output proportional to the position of the bubble in two orthogonal axes. These voltages were recorded on a two channel Rustrak chart-recorder with bias-steppers which gave an effective increased chart width². The system was powered by alkaline-manganese cells, and to conserve power the tiltmeter was run for three 1-min intervals in every hour. Sampling intervals were timed by an Accutron clock and the time scale of the record was derived by dividing the length of the record by the total number of days elapsed.

The tiltmeter was installed in a borehole 1.5 m deep in ash (Fig. 2), 3 km south of the Central Crater. The instrument was levelled using an oscilloscope with its horizontal and vertical inputs connected to the tiltmeter outputs; the top

of the tiltmeter tube was then moved manually to centralise the spot on the screen³.

On January 30, 1974 a small flank eruption began on the western side of Etna⁴, and during two eruptive periods (January 30–February 16 and March 11–29) produced two small pyroclastic cones and approximately $6 \times 10^6 \text{ m}^3$ of lava (Fig. 2). The correlation between the tilt record and this eruptive cycle is clear, detailed and apparently free of spurious effects. The following volcanological interpretation is based on the assumption that a positive change in the radial component indicates inflation of the summit region. The records of both the radial and tangential components of tilt during the 10 months of monitoring show very similar patterns of change (Fig. 3).

The tilt record of the period before January 1974 is very quiet, but about three weeks before the eruption (the two phases of which are represented by 1–2 and 3–4 in Fig. 3), the summit inflated and a small but regular fluctuation of tilt began. Two weeks before the eruption started the radial component recorded a series of very sharp though small deflations (Fig. 3ci) which gave way to a series of large amplitude fluctuations (Fig. 3cii) up to and immediately following the start of eruptive activity. The onset of the initial deflations corresponds closely with the start of the seismic activity that was felt at the volcano, and is interpreted as an expression of the initiation of fracture propagation from the magma reservoir at the summit to the eruption site on the western slopes. The large amplitude tilt fluctuations (Fig. 3cii) are cycles of rapid inflation followed by deflation of the summit and could represent the pulses of magma moving into the flanks of the volcano. Careful examination of the record shows that in most cases deflation was more rapid than the inflation, particularly for the tangential component. It may be possible

Fig. 1 Diagrammatic cross section of the tiltmeter installation.

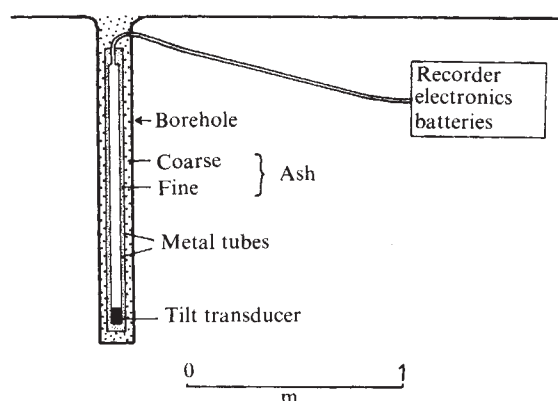
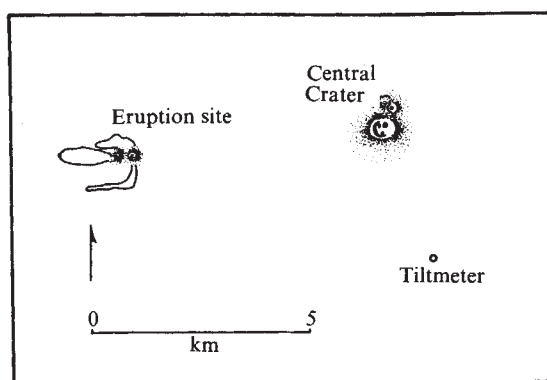


Fig. 2 Sketch map showing the location of the tiltmeter with respect to the eruption site and the Central Crater.



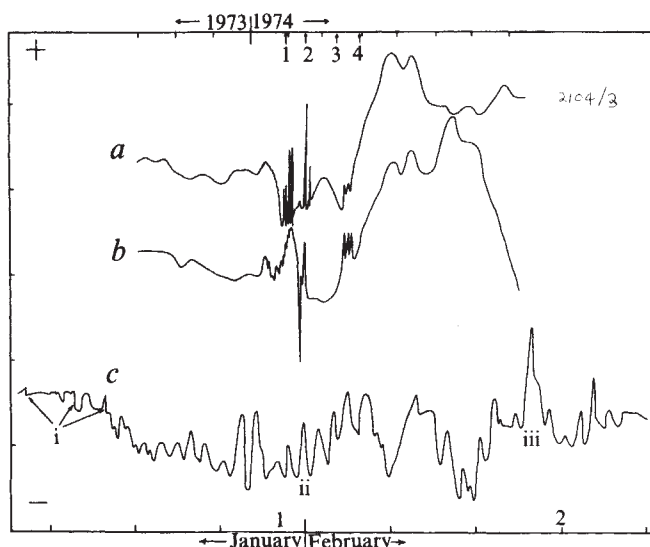


Fig. 3 Tiltmeter record for the period September 28, 1973–August 8, 1974. *a*, *b*, Smoothed traces of the record (sampled at weekly intervals), for the whole period, indicating the tilt variations in directions radial (*a*) and tangential (*b*) to the Central Crater at the summit of Etna. *c*, The non-smoothed trace of the radial component during and just before the first phase of the eruption. The scale of the abscissa for the two curves *a* and *b* is at monthly intervals, for *c* at 5-d intervals. The interval of the ordinate is 50 mrad (equivalent to a change in elevation of 5 cm km⁻¹), and the positive sense of tilt change shown is summit up (*a*), and ENE up (*b*).

to correlate these magma pulses with specific events in the vent activity. The particularly large tilt changes just before the end of the first phase of activity (Fig. 3ciii) coincided with a brief rejuvenation of effusive activity which produced three new lava flows⁴.

A period of calm characterised the tilt record for the interval between the two eruptive episodes during which the summit remained deflated. The degree of tilt that marked the start of the second phase was relatively minor compared with that of the first phase and is interpreted as indicating that the magma moved from the summit along the same conduit as that formed during the previous activity. The marked decline in vent activity and the rapid, smooth summit inflation on March 23 pre-saged the closing of the conduit and the end of the eruption.

The summit inflated throughout April and early May and then remained stable to the end of the record. The gradual tilt change in the tangential component during July and August 1974 cannot be interpreted as yet. The sense of this change—west(eruption) side down when the summit rose and vice versa—is unexpected, but could be because deflation of the western slopes complemented the inflating summit⁶.

The most striking feature of the record is the consistent frequency of the tilt fluctuations—one fluctuation a day—during and just before the eruption; a feature that is not evident during the non-eruptive periods. It suggests some non-volcanic causal mechanism such as, possibly, earth tides.

Since October 1971, precise electromagnetic measurements of distance have been made periodically on a network of 19 permanent benchmarks arranged in 2 concentric rings around the summit of the volcano (Fig. 4). Early surveys were made with the National Physical Laboratory Mekometer III prototype⁸ but since 1973 a Hewlett-Packard 3800B has been used.

Up until June 1973 the measurements showed changes of up to 310 mm in the lengths of the lines on the summit network. They are interpreted as showing that the summit region was inflating elastically⁷, although the Mogi model⁸—used to explain the deformation of Kilauea⁹—does not agree with the observations. The model which best describes the observations is that in which a vertical cylinder of infinite length but finite diameter expands in an elastic half-medium^{7,10}. The displacements of all of the points in the half-medium are inversely

proportional to the square of their distance from the cylinder axis and are directed radially outwards from it. The length changes observed up until June 1973 gave excellent best-fit agreement with the changes which could be expected were such a cylinder 200 m in diameter located beneath the North-east Crater (Fig. 4). Exceptions to this model occur on the lines involving stations 9, C1 and 13 (Fig. 4) which are situated on very recent flows and seem to have moved downslope after the apparent solidification of the lava.

The changes observed on the summit network between June 1973 and August 1974 (Fig. 4) do not fit such a simple model as the earlier changes.

Theoretical models

The model which best fits these results involves two cylinders one expanding and situated as before beneath the North-east Crater, and the other—of the same diameter—contracting and centred on the Chasm (the figures given by the model are shown in brackets in Fig. 4). The contracting cylinder would not affect the lines on the eastern side of the NE–SW trending fissure, the most important tectonic feature of the summit area¹¹. Anomalous movements again affect stations 9, C1 and 13, and the probable displacement vectors are shown in Fig. 4. Similarly, station 12 seems to have moved anomalously, though this is not understood.

The complexity of this model cannot be denied, but the volcanological interpretation of the cylinders is simple.

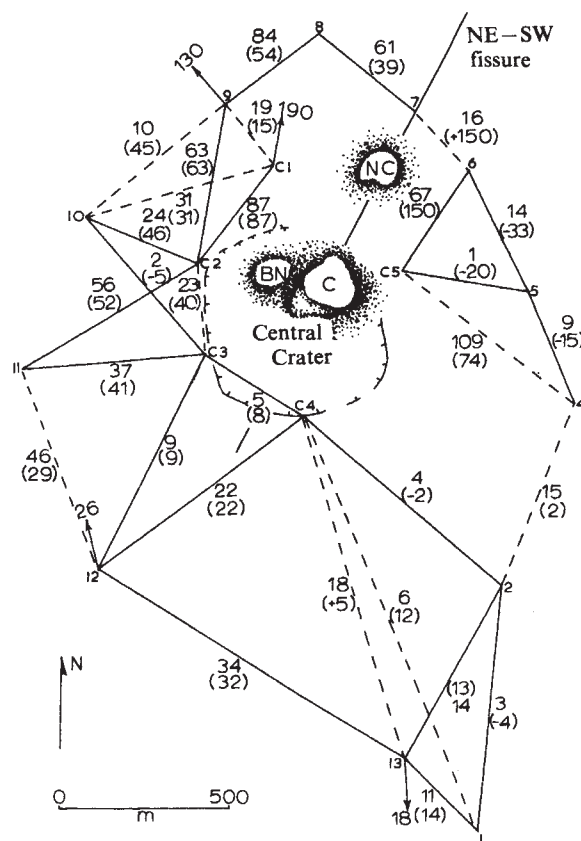


Fig. 4 The summit region of Mount Etna. C, The Chasm; BN, the Bocca Nuova—both of which are surface expressions of the Central Crater; NC, the North-east Crater. Geodetic benchmarks shown by small numbers. Solid lines, distances that increased; broken lines, those that decreased; unbracketed numbers, the observed changes of distances (mm) from June 1973 to August 1974. Bracketed numbers, changes (mm) expected from the model described in the text. Displacement vectors for the four anomalous stations are also shown.

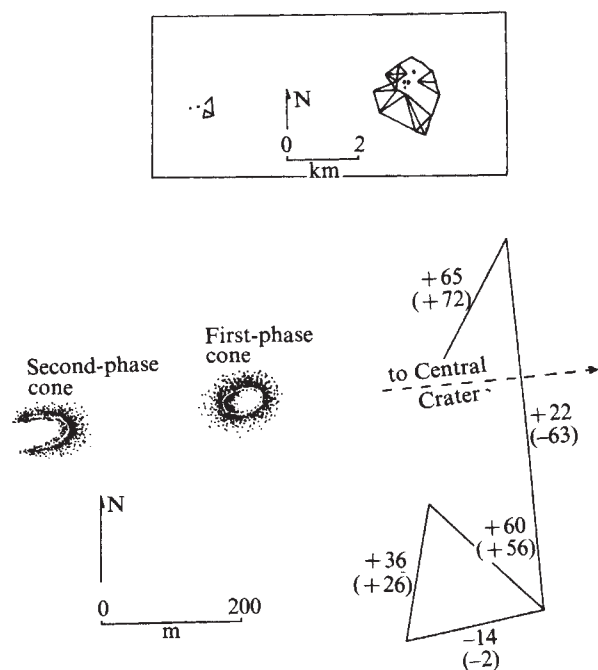


Fig. 5 The site of the January-March, 1974 eruption on the western flank of Etna, showing the first phase (January-February) cone, the second phase (March) cone, and the geodetic benchmarks. Unbracketed numbers, distance changes (mm) observed between February and August 1974; bracketed numbers, changes of length (mm) expected from the model described in the text. Inset, geography of the two networks.

The continuous effusive activity which characterised the North-east Crater for many years stopped abruptly during the 1971 eruption¹¹. It thus seems that before 1971 the North-east Crater had acted as a safety valve to the continuous movement of magma from below but that since then pressure has built up beneath a blockage in the conduit.

In contrast, the contracting cylinder part of the model indicates the reverse process; the decrease of pressure on the western side of the Central Crater, caused by the removal of magma from the conduit beneath the Chasm.

During the February phase of the 1974 eruption precise distance measurements were made on a small network of benchmarks in the immediate vicinity of the eruption site; they were repeated in August 1974. The changes between the measurements are shown in Fig. 5 as unbracketed numbers. A contracting cylinder model like that applied to the Chasm,

but of 50m diameter, situated beneath the first-phase cone agrees quite well with the observed changes. This can be improved, however, by including the influence of a horizontal cylinder which joins the first-phase cone to the Central Crater and which contracts by one-fifth the amount of the vertical one. The observed and expected changes of line 1-3 disagree, however (Fig. 5).

How the models fit

The vertical cylinder could represent the first phase vent, and the horizontal cylinder the feeder conduit from the Central Crater to the eruption. The contraction of the cylinders indicates the decrease of magmatic pressure which accompanied the cessation of effusive activity. The anomalous change in length of line 1-3 indicates that the feeder conduit (which passes directly beneath the line) widened inelastically during the second phase of the eruption.

The deflation of the western side of the summit region and of the horizontal cylinder used to model the deformation to the east of the eruption site suggests strongly that the eruption was fed by a conduit (dyke?) from the Central Crater. This view is endorsed by the evidence of a narrow line of micro-earthquakes along the proposed path of the conduit during the activity (G. Luongo, personal communication) and by the sympathetic behaviour of the Bocca Nuova¹² which suggests that the conduit originated there.

The 1974 eruption seems to have relieved the stress on the western side of the Central Crater but not that to the north around the North-east Crater; the continued inflation in that area must make it a likely place for renewed activity. In fact, since the last measurements were made quiet effusive activity has broken out at the foot of the North-east Crater (October 1974).

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Magnetic field in the intergalactic region

Jacques P. Vallée*

David Dunlap Observatory, University of Toronto, Canada

An analysis of the redshift dependence of the rotation measures (RM) and of the intrinsic position angles of the polarised radiation of radio galaxies and QSOs is made. The results place an upper limit of 10 rad m^{-2} to the observed RM, coming from the contribution of the magnetic field in the intergalactic region.

FARADAY rotation in a plasma produces a rotation of the plane of linear polarisation of the radio emission, the rotation increasing proportionately to the square of the wavelength. The proportionality factor is called the rotation measure (RM), and depends on the product of electron density, magnetic field, and path length inside the plasma. The plasma in the radio source gives rise to the source RM. Any intergalactic plasma will also contribute; the extragalactic RM is defined here as the observed RM minus the contribution from our

* Present address: Sterrewacht, Huygens Laboratory, Leiden 2405, The Netherlands.

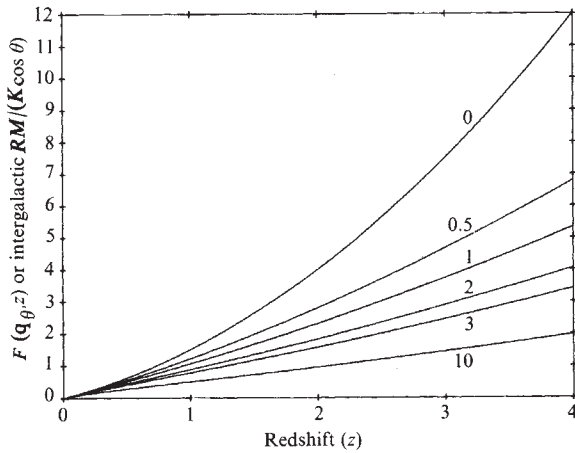


Fig. 1 The redshift dependence of $F(q_0, z)$ for various values of the cosmological deceleration parameter q_0 . Numbers on curves indicate values of q_0 .

Galaxy. It follows that, setting z_s as the source redshift,

$$\text{RM}(\text{observed}) = \text{RM}(\text{our Galaxy}) + \text{RM}(\text{extragalactic}) \quad (1)$$

$$\text{RM}(\text{extragalactic}) = \text{RM}(\text{intergalactic}) + \frac{\text{RM}(\text{source})}{(1+z_s)^2} \quad (2)$$

Values of $\text{RM}(\text{observed})$ and $\text{RM}(\text{extragalactic})$ will be reported elsewhere by myself and P. P. Kronberg. There are two possible ways in which a uniform component of an assumed intergalactic magnetic field can make a contribution to the extragalactic RM of a distant radio source.

If the smooth intergalactic density of thermal electrons throughout the observable Universe is sufficiently large, then each volume element along the line of sight to the radio source will contribute to the extragalactic RM, weakened by the factor $(1+z)^2$, where z is the redshift of the volume element. So if the lines of sight to two QSOs at different redshifts are very close to each other, they will have a different amount of their extragalactic RM coming from the intergalactic magnetic field.

If the intergalactic electron density close to a radio source is much larger than the smooth component permeating the observable universe, then the intergalactic magnetic field close to each radio source will contribute a part to RM, but weakened by the factor $(1+z_s)^2$, where z_s is the radio source redshift.

A significant contribution from a random intergalactic magnetic field would contradict the evidence of the least depolarisation occurring with the largest linear and angular sizes of radio sources, as shown by Strom^{1,2}.

The uniform model

Such a contribution to the extragalactic RM coming from any homogeneous component of an intergalactic magnetic field in a uniform model of the universe is given by:

$$\text{RM}(\text{intergalactic}) = \int_0^{R_{\text{source}}} A \cos \theta n_e(R) B(R) (1+z)^{-2} dR \quad (3)$$

where: $A = 8.1 \times 10^5 \text{ rad m}^{-2} \text{ cm}^3 \text{ gauss}^{-1} \text{ pc}^{-1}$; n_e = electron density (cm^{-3}); B = magnetic field (gauss); θ = angle between direction of B and line of sight; z = redshift of volume element at R ; and dR = element of path length for the radiation coming from the source.

From the field equations³ for zero-pressure models and

$$\Lambda \equiv 0, \quad dR = -cdt = cH_0^{-1} dz(1+z)^{-2}(1+2q_0z)^{-\frac{1}{2}} \quad (4)$$

where c = speed of light; H_0 = Hubble constant; t = cosmic time; and q_0 = deceleration parameter of the Universe.

In a homogeneous universe, it is plausible to use the assumptions that:

$$\begin{aligned} \text{mean matter density } \rho &\sim (1+z)^3 \\ \text{mean electron density } n_e &\sim \rho \sim (1+z)^3 \\ \text{magnetic field } B &\sim \rho^{2/3} \sim (1+z)^2 \text{ "frozen-in"} \end{aligned}$$

Integrating equation (3):

$$\text{RM}(\text{intergalactic}) = K \cos \theta F(q_0, z_s) \quad (5)$$

where $K = A n_{e0} B_0 (c/H_0)$

and

$$F(q_0, z) = [(1+2q_0z)^{3/2} + (6q_0-3)(1+2q_0z)^{1/2} + (2-6q_0)]/6q_0^2$$

and the subscript zero refers to the present epoch. An estimate of the numerical value of the quantity K is about 5 rad m^{-2} (assuming $n_{e0} = 10^{-6} \text{ cm}^{-3}$; $B_0 = 10^{-9} \text{ gauss}$; $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$). The function $F(q_0, z)$ is plotted in Fig. 1 for $0 \leq z \leq 4$, and for q_0 values of 0, 1/2, 1, 2, 3 and 10. $F(q_0, z)$ varies between 0 and 12 for radio sources in this range. (A mean density of the Universe of $3 \times 10^{-30} \text{ g cm}^{-3}$ corresponds to $n_e \sim 2 \times 10^{-6} \text{ cm}^{-3}$, assuming 100% ionisation and 100% hydrogen.)

I have used a numerical technique to fit the equation:

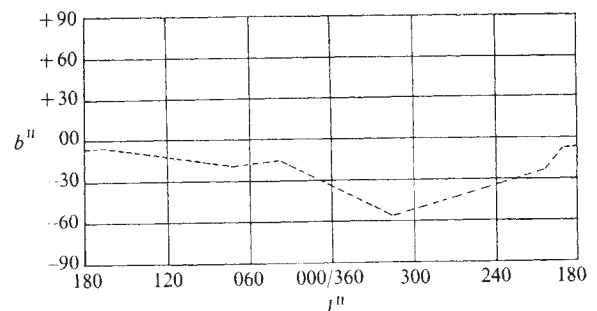
$$\text{RM}(\text{extragalactic}) = KK F(q_0, z) + A(3) \pm A(4)/(1+z)^2 \quad (6a)$$

to the data where $KK \equiv K \cos \theta \equiv A(1)$ (rad m^{-2}) and $q_0 \equiv A(2)$. $A(3)$ is a constant residual galactic contribution (rad m^{-2}) and $A(4)$ is the average positive source RM (rad m^{-2}). Because of the smoother magnetic field below the galactic plane⁴ I used as input data the extragalactic RMs of 44 QSOs and galaxies with known redshift in approximately half of the South Galactic Hemisphere, as will be discussed elsewhere (J. P. V. and P. P. Kronberg, unpublished).

This chosen region (Fig. 2) lies between the dashed line and the South Galactic Pole. It was arrived at by fitting a slab model to represent the galactic magnetic field contribution to the observed RM of discrete radio sources. In so doing, the RM with large absolute values ($> 150 \text{ rad m}^{-2}$) were removed, because of their effect on the least squares fitting technique used. We also selected sources with a redshift greater than 0.054 to eliminate unidentified galactic sources as well as to take advantage of the Doppler effect on source RM, enabling the galactic (local) contribution to RM to become more significant. The best fitted slab is not necessarily parallel to the galactic plane, and sources not seen through the slab were not used to determine the slab parameters. The boundary defining the input sources actually used in the best fitted slab corresponds to the dashed line in Fig. 2, enclosing the South Galactic Pole.

For all galaxies and known QSOs below the dashed line with observed RM, the galactic contribution to RM was computed and removed. First, assuming that $\cos \theta = \text{constant}$ for the redshifted sources below the dashed line, it was found that q_0 varied significantly when using different selection criteria on the input data.

Fig. 2 Projection of the sky in galactic coordinates, in which the region of the South Galactic Hemisphere studied in the two models discussed in the text is shown below the dashed line.



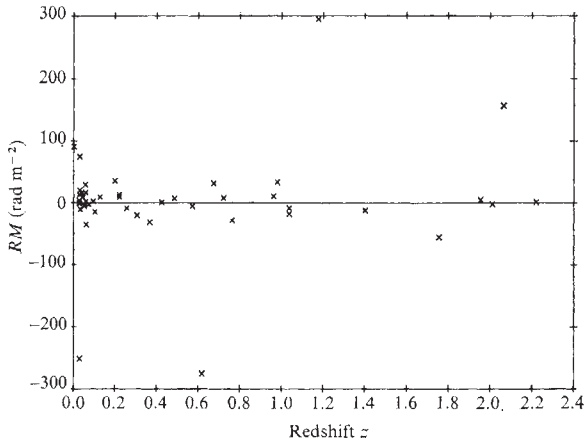


Fig. 3 Plot of the extragalactic rotation measures as a function of the redshift z for the 44 QSOs and galaxies studied in the two models.

So the approximation $\cos\theta = \text{constant}$ was rejected and θ computed for each radio source (assuming a given direction in the sky for the intergalactic magnetic field—see below) with as input data the extragalactic RM divided by $\cos\theta$. The fitting function used was

$$RM(\text{extragalactic})/\cos\theta = KF(q_0, z) \quad (6b)$$

where K and q_0 are to be fitted to the input data.

Many trials were made, using three different directions for the assumed intergalactic magnetic field. Only a direction towards the spiral arm magnetic field ($I^{\text{II}} = 90^\circ$) gave results that were not changed significantly when different selection criteria for the input data were used. This can perhaps be explained by a small residual contribution (or overcorrection) from the magnetic field in the galactic spiral arm.

As Fig. 3 shows, the sample contains about an equal number of sources with positive and negative values of extragalactic RM. If there is a sufficiently large contribution from the intergalactic region, then the quantity on the left hand side of equation (6b) should be of the same sign for all the radio sources (except for strong source RM). A plot of this quantity as a function of redshift also shows, however, about an equal number of sources with positive and negative values.

So an increase of extragalactic RM with increasing redshift seems ruled out within the errors permitted by the data. A useful upper limit to the extragalactic RM from the uniform model would be about 10 rad m^{-2} (Fig. 3). This value can be halved if a small residual galactic contribution is accepted ($A(3)$ in equation (6a)). A plausible alternative to the uniform model would be to say that any real contribution from an intergalactic magnetic field would originate near the radio source, instead of in a region far away from a radio source where the mean matter density and the ionisation rate are both likely to be smaller. This is the clumpy model.

The existence of intergalactic material in clusters of galaxies has often been proposed. De Vaucouleurs *et al.*⁵ presented evidence for intergalactic extinction in the light of normal optical galaxies located close to the plane of the supergalaxy (centred on the Virgo cluster of galaxies). Miley *et al.*⁶ proposed that the “head-tail” radio galaxies represent the radio trails of the trajectories of active radio galaxies through a dense intergalactic medium within clusters of galaxies. In trying to evaluate the contribution to the source rotation measure coming from an ionised intergalactic plasma, a major problem arises because the geometrical distribution of this plasma and the actual position of the radio source in the cluster are not known *a priori*.

To facilitate the computations I shall make several assumptions. First, the existence of a uniform component of an

intergalactic field. Second, that the ionised intergalactic plasma, within several megaparsecs of a radio source, is denser than the possibly smooth component permeating clusters of galaxies or even the Universe. Third, a geometrical distribution originating in the following form:

Let a radio source at redshift z_s eject n_e electrons cm^{-3} up to a source distance $\Delta R/2$ perpendicular to the intergalactic magnetic field B (no limit in the direction parallel to B). A subsequent diffusion along the lines of B may occur to equalise n_e in this cylindrical model with the radio source on the axis.

Provided that the products of $n_e B$, and ΔR are within an order of magnitude for most of the radio sources studied, and that the overall angular area in the sky is sufficiently small, the slab model (see above) can test for the existence of the ordered component of an intergalactic magnetic field. The input data consist of the extragalactic RM transferred into the source frames by:

$$RM(\text{extragalactic}) = \frac{RM(\text{source}) + RM(\text{intergalactic})}{(1+z_s)^2} \quad (6c)$$

Table 1 Rotation measures in the source frames, after removal of the contribution from our Galaxy, for use in the clumpy model

Name	RA, dec.	RM (rad m ⁻²)
3C2	0003-00	-37
3C9	0017+15	-24
3C15	0034-01	-4
3C17	0035-02	+13
PKS	0038-02	+1394
PKS	0043-42	-6
PKS	0045-25	+91
3C29	0055-01	+10
PKS	0056-00	+22
3C33	0106+13	+2
3C37	0115+02	+88
PKS	0119-04	+38
PKS	0131-367	-1
3C47	0133+20	+1
3C48	0134+32	-60
PKS	0155-10	-719
PKS	0226-038	+1472
PKS	0237-23	+12
3C76.1	0300+16	-12
3C78	0305+03	+14
3C79	0307+16	-14
3C88	0325+02	+22
3C94	0350-07	+40
3C98	0356+10	+79
PKS	0403-13	-14
3C109	0410+11	-35
3C135	0511+00	+11
3C138	0518+16	-88
PKS	0521-36	-40
3C403	1949+02	+19
PKS	2058-28	+17
PKS	2115-30	+132
3C433	2121+24	-18
PKS	2135-14	+51
PKS	2209+08	+16
3C442	2212+13	-1
3C445	2221-02	+33
3C446	2223-05	-71
PKS	2230+11	-78
PKS	2247+11	+4
3C454	2249+13	-427
3C459	2313+03	+20
3C465	2335+26	-267
PKS	2356-61	+3

The same radio sources used in the uniform model were used for analysis of the clumpy model; $RM(\text{source}) + RM(\text{intergalactic})$ are listed in Table 1.

The best results occur when radio sources with known redshifts are separated according to redshift. QSOs at $z > 0.5$ and with $RM(\text{source})$ less than 150 rad m^{-2} yield a direction for a possible magnetic field that is almost parallel (within the errors) to the spiral arm magnetic field in the Southern Galactic Hemisphere. It is possible that not all the galactic contribution had been removed from the observed RM because of the idealised

model of a slab, this effect showing up at large z because of the Doppler shift affecting RM(source). Thus, for QSOs at intermediate and large z , the residual galactic contribution is as big or bigger than the extragalactic RM.

For radio sources at redshifts closer than 0.5 and with RM (source) less than 150 rad m⁻², QSOs and galaxies taken together or separately yield the best results for a magnetic field direction $l^{\text{II}} = 200^\circ$, $b^{\text{II}} = 0^\circ$, with an error of 40° in both coordinates. The parameter K is about -7 rad m⁻². If we assume an intergalactic electron density n_e close to a radio source in the range 10^{-4} to 10^{-5} cm⁻³, and a value for ΔR in the range 10^6 to 10^8 pcs, we find that this value for K corresponds to a field strength in the range 10^{-6} to 10^{-9} gauss. This is to be compared with the upper limit of about 10^{-7} gauss set by Zel'dovich⁷ on the basis of an isotropic cosmological model concerning nuclear reactions. But in view of the strong assumptions used in the clumpy model, one cannot state that this last result constitutes a proof for the existence of an intergalactic magnetic field.

The intrinsic position angle of linear radiation is determined by the orientation of the magnetic field inside a radio source. It is altered neither by the intervening medium between the radio source and the Earth, nor by the Doppler effect. Some theoretical models of radio galaxies and QSOs (see ref. 8) presuppose the existence of an intergalactic magnetic field in the region of space where a radio galaxy or a QSO occurs. These models have the rotational axis of a galaxy orthogonal to the direction of the intergalactic magnetic field. Magnetic

"tongues" erupt along the rotational axis, and radio emission commences with the ejection of relativistic electrons into the tongues, through the synchrotron process; it is polarised with the electric vector parallel to the direction of the intergalactic magnetic field (for young radio sources). According to Piddington's model⁸ neighbouring sources may therefore have similar E-vector orientations.

I have plotted the 114 redshifted QSOs and galaxies with known RM in the Southern Galactic Hemisphere⁹ on Mercator maps, each radio source in its proper redshift range. The redshift ranges were: $0 < z < 0.1$, $0.1 < z < 0.2$, $0.2 < z < 0.3$, $0.3 < z < 0.5$, $0.5 < z < 0.7$, $0.7 < z < 1.0$, $1.0 < z < 1.5$, $1.5 < z < 2.3$. The median number of sources on these plots was nine. A qualitative study of the intrinsic position angles shown on these plots in various redshift ranges failed to indicate any parallelism, even for neighbouring sources in the sky.

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A gene cluster in *Aspergillus nidulans* with an internally located *cis*-acting regulatory region

Herbert N. Arst Jr & Donald W. MacDonald

Department of Genetics, University of Cambridge, Milton Road, Cambridge CB4 1XH, UK

Work reported here on the fungus Aspergillus nidulans has provided the first definitive demonstration of operon-type organisation in an eukaryote genome. It has been shown that the prnA and prnB genes concerned with proline metabolism form a gene cluster with the regulatory region lying between the two putative structural genes prnA and prnB. Regulatory mutations (prn^d) probably leading to relief of carbon catabolite repression, map in between prnA and prnB and are cis-dominant with respect to both. The properties of these regulatory mutations and other findings suggest that carbon catabolite repression may be mediated by a negative control system in A. nidulans. This gene cluster is particularly interesting in view of its divergent orientation (with the regulatory region located in the centre of the operon) and for the fact that unlike the divergent operons known in prokaryotes, the divergent orientation is related to the way in which this particular operon may be regulated.

instances in which the clustered genes code for enzymes which form aggregates (refs 1 and 2, and D. J. Cove, H. N. Arst, and C. Scazzocchio, in preparation). None of the few clusters which have been reported has been demonstrated to contain an internal control region such as that described^{3,4} in the *argECBH* cluster of *Escherichia coli*, where transcription proceeds divergently^{5,6}. Indeed, there has been no convincing demonstration of a *cis*-acting control region associated with a fungal gene cluster at all.

In *Saccharomyces cerevisiae*, however, there are several reports of *cis*-dominant operator constitutive type mutations adjacent to negatively controlled structural genes⁷⁻¹⁰. In *Aspergillus nidulans* (D. J. Cove *et al.*, unpublished) and *Neurospora crassa*¹, the preponderance of positive control systems weighs heavily against the probability of obtaining *cis*-acting regulatory mutations. Initiator constitutive mutations should be rare and the more frequent initiator negative mutations will be difficult to distinguish from negative mutations in adjacent structural genes under their control. New and powerful selective techniques have resulted, however, from an extensive study¹¹ of nitrogen metabolite repression (ammonium repression) of the syntheses of enzymes involved in nitrogen metabolism in *Aspergillus nidulans*. The product of a gene designated *areA* is likely to be a positive control element which allows the synthesis of a large number of enzymes and permeases involved in nitrogen metabolism. One class of mutations, designated *areA'*, leads to loss of these enzymes and permeases and a

In the ascomycete fungi, one of the most genetically well characterised groups of eukaryotes, clustering of functionally related genes is relatively rare. It is generally confined to

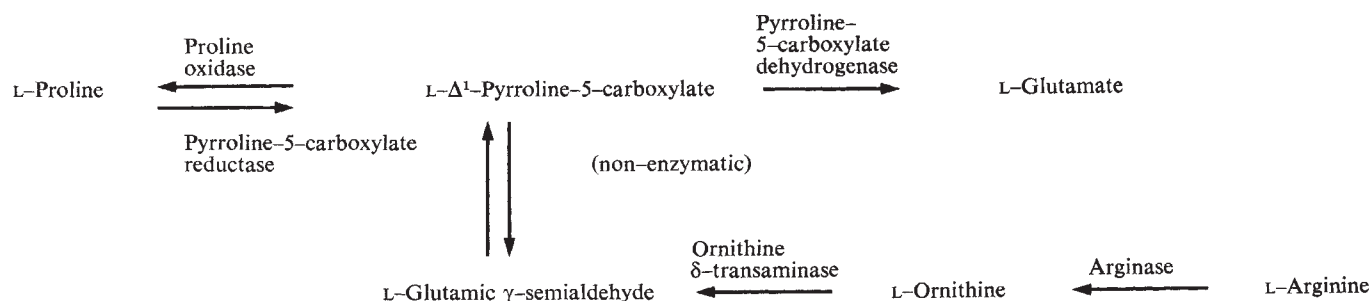


Fig. 1 Pathways of proline, ornithine, and arginine catabolism.

consequent inability to utilise a wide variety of nitrogen sources other than ammonium. Presumably, *areA*⁻ mutants lack a functional *areA* product. Given the pleiotropic nature of the *areA*⁻ phenotype, suppressor mutations affecting the regulation of a single enzyme or a group of enzymes having a common regulatory element can readily be detected. For example, mutations which relieve carbon catabolite repression have been obtained because they enable *areA*⁻ strains to utilise those nitrogen sources catabolised by carbon catabolite-repressible enzymes^{11,12}.

Here we present evidence that the regulation of proline catabolism in *A. nidulans* involves three distinct forms of control:

induction, nitrogen metabolite repression, and carbon catabolite repression.

Moreover, a putative structural gene for a proline permease is tightly linked to a putative structural gene for a component common to proline oxidase and Δ^1 -pyrroline-5-carboxylate dehydrogenase. Between these two genes is a region in which *cis*-acting regulatory mutations affect the expression of both.

Control of proline transport and catabolism

The relevant pathways¹³⁻¹⁷ of proline and ornithine catabolism are shown in Fig. 1. Control characteristics of proline transport,

Table 1 L-Proline transport by wild type and mutant strains

Relevant genotype or calculation	Specific transport activities using mycelia grown in media with							
	595 μ M uric acid				0.3% D-glucose as C			
	1% D-glucose as C		10 mM NH ₄ ⁺		595 μ M uric acid		10 mM NH ₄ ⁺	
	—	+	—	+	—	+	—	+
		5 mM L-proline		5 mM L-proline		5 mM L-proline		5 mM L-proline
Wild type	0.80	1.63	0.42	1.26	0.95	2.80	0.27	2.40
<i>prnA</i> -1	0.76	1.65	0.50	1.40	—	—	—	—
<i>prnB</i> -6	0.58	0.70	0.12	0.15	0.50	0.66	0.08	0.30
<i>prnA</i> -1 <i>prnB</i> -6	0.60	0.63	0.15	0.17	—	—	—	—
<i>prn</i> ^d -20	0.40	1.80	0.70	1.90	1.50	3.50	0.30	4.70
<i>prn</i> ^d -20 <i>prnB</i> -36	0.12	0.28	0.14	0.40	0.14	0.30	0.06	0.14
<i>creA</i> ^d -1	0.80	2.50	0.50	1.86	0.78	2.80	—	—
Corrected wild type	0.22	0.93	0.30	1.11	0.45	2.14	0.19	2.10
Corrected <i>prn</i> ^d -20	-0.18	1.10	0.58	1.75	1.00	2.84	0.22	4.40
Corrected <i>creA</i> ^d -1	0.22	1.80	0.38	1.71	0.28	2.14	—	—

Fourteen mutants unable to utilise proline as a nitrogen source were isolated after N-methyl-N'-nitro-N-nitrosoguanidine (NTG) mutagenesis²⁶ of a strain of genotype *yA*-2 *proA*-6 *puA*-2 (yellow conidial colour, proline-requiring, putrescine-requiring), using the putrescine starvation selection technique²⁷. On the basis of growth responses (Tables 4 and 5) and complementation tests, eight of these were designated *prnA*⁻ and the other six *prnB*⁻. *prnA*⁻ and *prnB*⁻ mutations are recessive and complement with each other in heterokaryons and diploids. Most further work has been done with *prnA*-1 and *prnB*-6. The tight linkage of *prn*^d mutations to *prnA* and *prnB* makes it impracticable to obtain *prn*^d *prnA*⁻ and *prn*^d *prnB*⁻ double mutants by recombination. Therefore, *prnA*-33, *prnB*-32, *prnB*-35, and *prnB*-36 were selected after NTG mutagenesis of a *yA*-2 *proA*-6 *puA*-2 *prn*^d-20 strain and *prnB*-50 was selected after NTG mutagenesis of a strain of genotype *proA*-6 *puA*-2 *prn*^d-21 *fwA*-1 (*fwA*-1 leading to fawn conidial colour) using the method outlined above. All of these *prnA*⁻ and *prnB*⁻ mutations are recessive and their locus designations have been confirmed by diploid complementation tests. Retention of the *prn*^d mutation has been demonstrated in every case by its recovery on outcrossing.

For uptake studies, outcrossed strains carrying *pabaA*-1 (*p*-aminobenzoate requirement) were used. The *prn*^d-20 *prnB*-36 strain also carries *fwA*-1. Otherwise, all strains are isogenic, apart from the specified genotype. Mycelia were grown for 21 h at 25 °C in appropriately supplemented shaken liquid minimal medium²⁸ containing either 0.3 or 1% D-glucose as carbon source and either 595 μ M uric acid or 10 mM NH_4^+ (as the (+)-tartrate) as nitrogen source. The medium was buffered to pH 6.5 using 10 mmol l⁻¹ orthophosphate (as the sodium salts). Where the medium was made 5 mM with respect to L-proline, this was done after 16 h growth. Mycelia were collected by filtration on to nylon mesh washed with sterile, proline-free medium at 25 °C, and resuspended in the appropriate fresh sterile growth medium without proline. Aliquots (1.0 ml) of this suspension were transferred to 50 ml Erlenmeyer flasks containing 9.0 ml of the respective growth media without proline. L-proline-U-¹⁴C was added to each flask so as to give a final concentration of 200 μ M at a specific activity of 125 mCi mol⁻¹. Flasks were incubated at 25 °C in an orbital shaker. Duplicate 5.0 ml samples were taken after 0 and 5 min incubation. Each sample was filtered on to a preweighed 2.5 cm diameter Whatman GF/A filter paper, washed with 10 ml ice-cold 200 μ M L-proline (unlabelled) followed by 10 ml ice-cold distilled water. The filters were dried for 3 h at 90 °C, weighed again, placed in 10 ml of toluene scintillant containing 4.0 g 2,5-diphenyloxazole and 40 mg 1,4-bis-(2-(5-phenyloxazolyl))-benzene l⁻¹, and counted in a Packard 3375 Tri-Carb Scintillation Counter. Uptake of L-proline was linear up to 20-30 min incubation and was completely inhibited by 5 mM KCN or 100 μ g ml⁻¹ 2,4-dinitrophenol. Zero time values have been subtracted from 5 min values to correct for nonspecific adsorption. Results are expressed in nmol L-proline per min per mg dry weight. Corrected values have been obtained by subtracting the activities present in the *prnB*-6 strain under each set of growth conditions from the values for the strain specified in the table. This correction eliminates the contribution of the minor proline permease (see below).

In addition to the major proline permease whose control is outlined in this paper, there is at least one minor proline permease. It is most clearly observed in mutants lacking the major permease (*prnB*⁻). This minor permease, which is responsible for the leakiness of *prnB*⁻ mutants, does not respond to induction by proline but is markedly inhibited or repressed by ammonium. This ammonium inhibition or repression is also apparent from the increased sensitivity of proline oxidase to ammonium repression in *prnB*⁻ strains (Table 2) as a result of inducer exclusion. Ammonium inhibition or repression of the minor permease is sufficient to account for the slight effect of ammonium on proline transport in the wild type seen above.

0.3% glucose was chosen for transport studies in preference to lower concentrations so as not to restrict the energy available for the uptake process itself, even though carbon catabolite repression is still evident at this concentration. A strain carrying *creA*^d-1, which relieves carbon catabolite repression of the syntheses of a number of enzymes^{11,12}, has been included as it also relieves carbon catabolite repression of proline transport.

Table 2 Proline oxidase activities of wild type and mutant strains

Relevant genotype	Relative activities of proline oxidase of mycelia grown in media with 595 µM uric acid as N									
	1% D-glucose as C					0.3% D-glucose as C			0.1% D-glucose as C	
	—	5 mM L-proline	5 mM L-proline + 10 mM NH ₄ ⁺	5 mM L-ornithine	5 mM L-ornithine + 10 mM NH ₄ ⁺	—	5 mM L-proline	5 mM L-proline + 10 mM NH ₄ ⁺	5 mM L-proline	5 mM L-proline + 10 mM NH ₄ ⁺
Wild type	4.0	100	27	41	2.0	7.5	130	44	6.0	180
<i>prnA</i> -1	0.0	0.0	0.0	0.0	0.0	—	—	—	—	0.0
<i>prnB</i> -6	5.0	47	6.0	48	3.0	6.0	96	15	3.0	108
<i>prn</i> ^d -20	6.6	111	63	56	1.7	18	165	88	21	190
<i>prn</i> ^d -20 <i>prnA</i> -33	0.0	0.0	0.0	0.0	0.0	—	—	—	—	—
<i>prn</i> ^d -20 <i>prnB</i> -36	1.7	42	12	62	0.0	16	98	3.0	6.0	81

All strains carry *pabaA*-1, and the *prn*^d-20 *prnB*-36 strain also carries *fwA*-1. Otherwise, all strains are isogenic, apart from the specified genotype. Mycelia for enzyme assays were grown, collected and extracted as described previously^{28,29} in appropriately supplemented shaken liquid minimal medium with D-glucose at the specified concentrations as carbon source and 595 µM uric acid as nitrogen source. Mycelia were grown for 21 h at 25 °C and, where indicated, 5 mM L-proline or L-ornithine (as the monohydrochloride) with or without 10 mM NH₄⁺ (as the (+)-tartrate) was added after 16 h growth. Cell-free extracts were prepared in 100 mM Tris-HCl buffer pH 7.5 containing 500 mM sucrose. The crude homogenates were centrifuged at 12,000g for 10 min, and the supernatants taken for enzyme assay.

Proline oxidase (EC 1.4.3.2) assays were carried out in 1 cm cuvettes in a double beam spectrophotometer, at 30 °C. The assay mixtures contained in a total volume of 3.0 ml: 200 µmol Tris-HCl pH 8.5, 10 µmol 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride, 1.3 µmol N-methylphenazonium methosulphate, 42 nmol FAD, and 0.1 ml enzyme extract containing 200–500 µg protein. The reaction was started by adding 150 µmol L-proline. The blank cell contained all components of the assay with the exception of proline and the increase in absorbance at 500 nm was measured. Soluble protein in extracts was determined by the Biuret method³⁰, and results were expressed as a percentage of the specific activity of the wild type when grown on 1% glucose and induced with 5 mM L-proline.

Induction of proline oxidase by ornithine requires its conversion to glutamic γ-semialdehyde (or Δ¹-pyrroline-5-carboxylate) as it does not occur in strains lacking ornithine δ-transaminase whereas proline induction occurs normally (data not shown).

proline oxidase, and Δ¹-pyrroline-5-carboxylate (P5C) dehydrogenase in the wild type can be deduced from Tables 1, 2, and 3, respectively. All three activities are inducible. Proline transport and proline oxidase at least are carbon catabolite-repressible (carbon catabolite repression of P5C dehydrogenase has not yet been investigated). But, whereas proline oxidase and P5C dehydrogenase are ammonium-repressible, proline transport clearly is not.

It should be noted that ammonium represses proline oxidase even when there is little or no carbon catabolite repression (at 0.1% glucose) (Table 2). Thus carbon catabolite repression is probably not a prerequisite for nitrogen metabolite repression where the two forms of control coexist. Rather, these data suggest that the two forms of control operate more or less independently of each other.

Mutants unable to catabolise proline

Selection of *prnA*[−] and *prnB*[−] mutations is described in the legend to Table 1. *prnA*[−] strains lack both proline oxidase and P5C dehydrogenase (Tables 2 and 3) but are normal for proline

transport (Table 1) in agreement with their *in vivo* behaviour (Tables 4 and 5). *prnB*[−] strains are defective in proline transport (Tables 1 and 5). Consistent with this uptake defect, they show somewhat reduced levels of proline oxidase and P5C dehydrogenase when proline, but not ornithine, is used as inducer (Tables 2 and 3).

Regulatory mutations affecting proline catabolism

areA⁺ and *prn*^d mutations have been described previously¹¹. *prn*^d mutations are locus-specific suppressors of *areA*⁺ mutations, uniquely permitting utilisation of proline as nitrogen source.

prn^d-20 does not markedly affect uninduced levels of proline uptake, proline oxidase, or P5C dehydrogenase (Tables 1, 2 and 3). It does, however, result in derepression. For proline uptake, which is not ammonium-repressible, *prn*^d-20 is probably best interpreted as conferring carbon catabolite derepression. This is most clear in the presence of a partially carbon catabolite-repressing glucose concentration such as 0.3% (see

Table 3 Δ¹-Pyrroline-5-carboxylate dehydrogenase activities of wild type and mutant strains

Relevant genotype	Relative activities of P5C dehydrogenase of mycelia grown in media with 1% D-glucose as C + 5 mM urea as N			
	—	5 mM L-proline	5 mM L-proline + 10 mM NH ₄ ⁺	5 mM L-ornithine
Wild type I	2.7	100	25	—
Wild type II	0.0	115	20	25
<i>prnA</i> -1	0.0	0.0	—	—
<i>prnB</i> -6	0.0	76	—	—
<i>prn</i> ^d -20	0.0	103	56	—

The wild type I, *prnA*-1, *prnB*-6, and *prn*^d-20 strains carry *pabaA*-1, and the wild type II strain carries *biA*-1, a biotin auxotrophy, but is *pabaA*⁺. Otherwise, all strains are isogenic, apart from the specified genotype. Experimental details are given in the legend to Table 2 with the following modifications and additions: Cell-free extracts were prepared in 100 mM Tris-HCl buffer pH 7.5 containing 20% (v/v) glycerol. Pyrroline-5-carboxylate dehydrogenase (EC number not assigned) assays were carried out in 1 cm cuvettes in a double beam spectrophotometer at 25 °C. The assay mixtures, modified after Strecker³¹, contained in a total volume of 3.0 ml: 250 µmol Tris-HCl pH 8.5, 3.0 µmol NAD, and 0.1 ml of enzyme extract. The reaction was started by adding 6 µmol DL-Δ¹-pyrroline-5-carboxylic acid, synthesised as described by Strecker³². The blank cell contained all components except pyrroline-5-carboxylate, and the increase in absorbance was measured at 340 nm. Results are expressed as a percentage of the specific activity of wild type I (*pabaA*-1) when grown on 1% glucose and 5 mM urea and induced with 5 mM L-proline.

legend to Table 1). From the enzyme data it is not possible to deduce whether derepression of *prn*^d-20 is the consequence of carbon catabolite derepression or ammonium derepression or both since the relative contributions of the two forms of repression cannot be clearly distinguished (see ref. 11). But, by analogy with the permease results, we favour a model in which *prn*^d-20 relieves carbon catabolite repression of the proline catabolic enzymes. Mutations relieving carbon catabolite repression of the enzymes of proline degradation have been reported in *Salmonella typhimurium*¹⁸.

Epistasis relationships

prn^d mutations do not suppress *prnA*⁻ or *prnB*⁻ mutations (Tables 1, 2, 4, and 5). Therefore *prn*^d mutations affect the regulation of the proline oxidase and the P5C dehydrogenase which are missing in *prnA*⁻ strains and of the proline transport system which is missing in *prnB*⁻ strains.

both the *prnA* and *prnB* genes. Diploids of the following (relevant) genotypes were constructed:

$$\begin{array}{cc} \frac{areA^{-1} prn^{d-20} prnA-33}{areA^{-1} +} & \frac{areA^{-1} prn^{d-20} prnB-32}{areA^{-1} +} \\ \\ \frac{areA^{-1} prn^{d-20} prnB-35}{areA^{-1} +} & \frac{areA^{-1} prn^{d-20} prnB-36}{areA^{-1} +} \end{array}$$

All are unable to utilise proline as nitrogen source. (The presence of *prn*^d-20 in *areA*⁻¹ *prn*^d-20 *prnA*(or *B*)⁻ strains was verified by crossing to an *areA*⁻¹ strain and recovering progeny able to grow on proline as nitrogen source (genotype of progeny *areA*⁻¹ *prn*^d-20).) Diploids of (relevant) genotype (*areA*⁻¹ *prn*^d-20 +)/(*areA*⁻¹ + *prnA*-1) and (*areA*⁻¹ *prn*^d-20 +)/(*areA*⁻¹ + *prnB*-6) utilise proline as well as (*areA*⁻¹ *prn*^d-20)/(*areA*⁻¹ +) diploids.

Table 4 Growth responses of wild type and mutant strains

Relevant genotype	Growth on media containing					
	1% D-glucose as C+ L-proline	as N at 5 mM L-ornithine	10 mM NH ₄ Cl as N L-glutamate	as C+ at 50 mM L-proline	as N at 50 mM L-ornithine	as C+ at 50 mM L-glutamate
Wild type	++	++	++	+	+	+
<i>prnA</i> ⁻	-	±	++	-	-	+
<i>prnB</i> ⁻	±	++	++	±	+	+
<i>prn</i> ^d	++	++	++	+	+	+
<i>otaA</i> ⁻	++	-	++	+	-	+
<i>areA</i> ^r	-	-	-	+	+	+
<i>prnA</i> ⁻ <i>prnB</i> ⁻	-	±	++	-	-	+
<i>prn</i> ^d <i>prnA</i> ⁻	-	±	++	-	-	+
<i>prn</i> ^d <i>prnB</i> ⁻	±	++	++	±	+	+
<i>areA</i> ^r <i>prn</i> ^d	+	-	-	+	+	+
<i>prnA</i> ⁻ <i>otaA</i> ⁻	-	-	++	-	-	+
<i>areA</i> ^r <i>prn</i> ^d <i>prnA</i> ⁻	-	-	-	-	-	+
<i>areA</i> ^r <i>prn</i> ^d <i>prnB</i> ⁻	-	-	-	±	+	+

Growth tests were carried out at 37 °C on appropriately supplemented solid minimum medium²⁸. L-ornithine was added as the monohydrochloride, and L-glutamate was added as the monosodium salt. The *otaA*⁻ allele used here was *otaA*-2, selected as resulting in reduced utilisation of arginine as nitrogen source (L. M. Palmer and H. N. Arst, unpublished). It is phenotypically identical to its allele *otaA*-1, leading to loss of ornithine δ-transaminase, selected as preventing endogenously channelled or exogenously supplied arginine and ornithine from supplementing proline auxotrophies resulting from blocks between L-glutamate and L-glutamic γ-semialdehyde (Δ²-pyrroline-5-carboxylate) in the proline biosynthetic pathway^{13,14}. The alternative pathway of proline biosynthesis from arginine and ornithine is shown in Fig. 1. Gene symbols other than *otaA* are introduced in the text.

One noteworthy relationship is the lack of epistasis of *areA*^r mutations to *prnB*⁻ mutations with respect to supplementation of proline auxotrophies (Table 5). When ammonium is present to repress or inhibit the residual proline uptake present in *prnB*⁻ strains (legend to Table 1), *prnB*⁻ mutations prevent supplementation of proline auxotrophies by limiting levels of proline. *areA*^r mutations do not have this effect. *proA*⁻ *areA*^r *prnB*⁻ triply mutant strains show an intermediate growth response between that of *proA*⁻ (or *proA*⁻ *areA*^r) and *proA*⁻ *prnB*⁻ strains (Table 5). This is unlikely to be an allele-specific phenomenon because it occurs with *areA*^r-1, *areA*^r-2, and *areA*^r-19 (formerly designated *amdT*-19^{11,19}). Therefore the *areA*^r phenotype does not include loss of the *prnB* function under conditions in which it completely abolishes utilisation of proline as a nitrogen source. Thus, the lack of ammonium repression of the *prnB* permease is confirmed *in vivo* by differences in *areA*^r and *prnB*⁻ phenotypes and the lack of epistasis of *areA*^r to *prnB*⁻.

Cis-dominance of *prn*^d-20

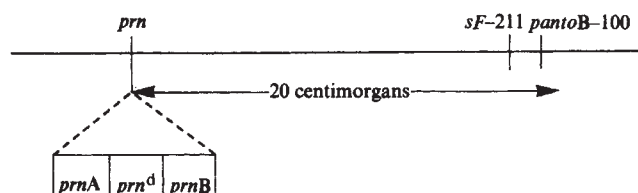
Diploids of (relevant) genotype (*areA*⁻¹ *prn*^d-20)/(*areA*⁻¹ +) utilise proline as a nitrogen source to an extent which is clearly intermediate between *areA*⁻¹ *prn*^d-20 haploids and *areA*⁻¹ *prn*⁺ haploids. *Cis-trans* tests show that the semi-dominance of *prn*^d-20 reflects the fact that it acts only in *cis* with respect to

Map positions

Haploidisation analysis²⁰ located both *prnA*-1 and *prnB*-6 to linkage group VII. Meiotic analysis showed them to be tightly linked (2–3 centimorgans). Preliminary work also showed that they are tightly linked to *prn*^d-20. Therefore crosses containing the following marker configurations were analysed: *prn*^d-20 *prnB*-32 × *prnA*-1; *prn*^d-20 *prnB*-35 × *prnA*-1; *prn*^d-20 *prnB*-36 × *prnA*-1; *prn*^d-20 *prnA*-33 × *prnB*-6; *prn*^d-21 *prnB*-50 × *prnA*-1. Proline-utilising progeny were isolated and tested for the *prn*^d phenotype. *prn*^d mutations can be scored in *areA*⁻¹ strains by methods described previously¹¹. In every case, proline-utilising progeny fell into both *prn*^d and *prn*⁺ classes. For example, among 64 proline-utilising progeny from a cross of configuration *prn*^d-20 *prnB*-35 × *prnA*-1, there were 27 *prn*^d-20 and 37 wild type strains. Insufficient numbers of progeny were analysed to warrant detailed presentation of quantitative results, but the qualitative results are unequivocal: *prn*^d-20 and -21 map between *prnA*⁻ and *prnB*⁻ mutations and do not lie markedly closer to one than to the other.

As the above crosses were also heterozygous for *pantoB*-100 (pantothenic acid auxotrophy), which maps about 20 centimorgans away, they showed that *pantoB*-100 is located on the *prnB* side of the *prn* cluster. To orientate the *prn* cluster on the linkage group VII map, its position with respect to sF-211 (a block beyond sulphite on the sulphate assimilation pathway²¹), closely linked to *pantoB*-100 (ref. 22), was deter-

mined by screening for *prnA*-1 among *sF*⁺ *pantoB*⁺ progeny of a cross of marker configuration *sF*-211 × *pantoB*-100 *prnA*-1. The linkage data can be summarised as follows:



otaA-2 (ornithine δ -transaminaseless¹³) is also in linkage group VII (L. M. Palmer, unpublished), but it recombines freely with mutations in the *prn* cluster.

prn^d Mutations are not allelic to *prnA*⁻ or *prnB*⁻ mutations

There is compelling evidence that *prn*^d mutations cannot be allelic either to *prnA*⁻ or to *prnB*⁻ mutations. Firstly, *prn*^d mutations affect both proline uptake and the enzymes proline oxidase and P5C dehydrogenase (Tables 1, 2 and 3). *prnA*⁻ mutations do not affect proline uptake. *prnB*⁻ mutations do not affect the levels of the two enzymes (except to an extent which is attributable to reduced inducer uptake when proline is used to induce).

Second, since *prn*^d mutations lack *trans* activity, they do not alter the structure of a diffusible product. Since *prnA*⁻ and *prnB*⁻ mutations complement each other, these mutations do alter the structure of diffusible products. Given that *cis*-acting regulatory regions are unlikely to overlap with structural genes, *prn*^d mutations cannot be allelic to either *prnA*⁻ or *prnB*⁻ mutations.

Gene regulation

We favour a model in which *prn*^d mutations define a distinct regulatory region located between two structural genes, *prnA* and *prnB*. Although it is clear that *prn*^d mutations do not occur in the *prnA* and *prnB* genes, we have not yet attempted to show directly that *prn*^d mutations do not alter the structure of the proline permease, proline oxidase, or P5C dehydrogenase. Unfortunately, preliminary attempts to obtain conditional *prnA*⁻ and *prnB*⁻ mutations, which might allow definitive identification of structural genes, have been unsuccessful (D. J. Cove and H. N. Arst, unpublished). There is no evidence that mutations in genes other than *prnA* can lead to loss of proline oxidase, but very recently a mutation whose

in vivo behaviour suggests that it eliminates P5C dehydrogenase has been obtained (H. N. Arst, unpublished). It has not yet been located.

A divergent orientation with an internal control region is an obvious way to organise two clustered genes (or sets of genes) having at least one control element in common and one control element not in common. Whereas both the *prnA* and the *prnB* products are probably subject to induction and carbon catabolite repression, only the *prnA* product is likely to be subject to nitrogen metabolite repression. As *prn*^d mutations affect the control of both *prnA* and *prnB* but do not significantly affect induction, they would seem to involve principally carbon catabolite derepression. Future work will be directed towards elucidating the induction process and, in the case of *prnA*, the mechanism of nitrogen metabolite repression.

An intriguing aspect of *prn*^d mutations is the high frequency with which they are induced (H. N. Arst, unpublished), suggesting that the target in the region between *prnA* and *prnB* where mutation can result in the *prn*^d phenotype is rather large. Perhaps *prn*^d mutations occur in a receptor site for a negative control element. There are other indications that carbon catabolite repression might be mediated by a negative control mechanism in *A. nidulans*: *creA*^d-1, leading to carbon catabolite derepression, is recessive¹², and *creA*^d mutations are not uncommon (C. Bailey and H. N. Arst, unpublished). The role of the *creA* gene in carbon catabolite repression has yet to be established, but these observations are consistent with its coding for a repressor which binds to sites such as that where *prn*^d mutations occur. Extensive attempts to obtain mutants simultaneously defective in the utilisation of several carbon sources have failed (C. Bailey and H. N. Arst, unpublished), which is consistent with the expected rarity of a super-repressed phenotype. Moreover, a negative model for carbon catabolite repression provides a rationale for the observation¹¹ that, among a number of nitrogen sources on which revertants of *areA*^r strains have been obtained, specific suppressor mutations have been selected only for proline and acetamide. These are the only two nitrogen sources whose utilisation by *areA*^r mutants can be achieved by the alleviation of carbon catabolite repression. Obtaining *cis*-acting regulatory mutations adjacent to *amdS*, the structural gene for acetamidase²³, is complicated by the occurrence of mutations in two unlinked regulatory genes, *amdR*^{11,24} and *amdA* (H. N. Arst, unpublished), suppressing *areA*^r mutations on acetamide. While revising this manuscript, we learned that Hynes²⁵ obtained a *cis*-dominant regulatory mutation tightly linked to *amdS*. He prefers to interpret his mutation as leading to increased inducibility, but his data do not eliminate the possibility that it leads to carbon catabolite derepression.

Table 5 Effects of various mutations on supplementation of proline auxotrophies

Relevant genotype	5 mM L-proline	Growth using supplementation by 250 μ M L-proline	5 mM L-ornithine or L-arginine
<i>proA</i> ⁻	++	+	++
<i>proA</i> ⁻ <i>prnA</i> ⁻	++	+	++
<i>proA</i> ⁻ <i>prnB</i> ⁻	++	-	++
<i>proA</i> ⁻ <i>prn</i> ^d	++	+	++
<i>proA</i> ⁻ <i>otaA</i> ⁻	++	+	-
<i>proA</i> ⁻ <i>areA</i> ^r	++	+	++
<i>proA</i> ⁻ <i>areA</i> ^r <i>prnB</i> ⁻	++	±	++
<i>proA</i> ⁻ <i>prn</i> ^d <i>prnB</i> ⁻	++	-	++
<i>proA</i> ⁻ <i>prnA</i> ⁻ <i>prnB</i> ⁻	++	-	++
<i>proA</i> ⁻ <i>areA</i> ^r <i>prn</i> ^d <i>prnB</i> ⁻	++	±	++
<i>proB</i> ⁻	++	+	++
<i>proB</i> ⁻ <i>prnB</i> ⁻	++	-	++
<i>proB</i> ⁻ <i>otaA</i> ⁻	++	+	-

Growth tests were carried out at 37 °C on appropriately supplemented solid minimal medium²⁸ with 1% D-glucose and 10 mM NH₄⁺ (as the (+)-tartrate) as C and N sources, respectively. *proA*-6 and *proB*-3 were utilised as the *proA*⁻ and *proB*⁻ alleles respectively. They are closely linked but non allelic mutations to proline auxotrophy and are blocked between L-glutamate and L-glutamic γ -semialdehyde (Δ^1 -pyrroline-5-carboxylate) on the proline biosynthetic pathway¹⁴. Gene symbols other than *proA* and *proB* are introduced in the text and the legend to Table 4.

Note that *proA*⁻ *areA*^r *prnB*⁻ triple mutants respond better to limiting proline levels (250 μ M) than *proA*⁻ *prnB*⁻ double mutants. This is probably the result of the lack of an ammonium-repressible ammonium transport system in *areA*^r strains¹¹, assuming that ammonium must enter the cell to inhibit or repress residual proline uptake.

Whatever the mechanism of carbon catabolite repression in *A. nidulans*, the nature of the effector involved is an open question. Neither cyclic AMP nor its N⁶-2'-O-dibutyl derivative seem to reverse carbon catabolite repression in any of several sensitive *in vivo* diagnostic systems (C. Bailey and H. N. Arst, unpublished). But there is no evidence that these compounds actually enter the cell, for they neither supplement adenine auxotrophies nor serve as nitrogen sources.

We believe this to be the first definitive demonstration of an operon-type structure (as opposed to simple tight linkage of functionally related genes) in an eukaryotic organism.

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Note added in proof: Recessive mutations eliminating P5C dehydrogenase whilst not reducing levels of proline transport or proline oxidase define another gene, *prnC*. *prnC*⁻ mutations are tightly linked to the other *prn* mutations, with the map, order being *prnA prnB prnC . . . pantoB*.

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Initiator constitutive mutation with an 'up-promoter' effect in *Aspergillus nidulans*

Herbert N. Arst Jr & Claudio Scazzocchio*

Department of Genetics, University of Cambridge, Milton Road, Cambridge CB4 1XH, UK

A mutation leading to strong constitutivity for a uric acid-xanthine permease in the lower eukaryote Aspergillus nidulans has been found to be tightly linked to the putative structural gene whose expression it controls in the cis configuration. In addition, it increases the maximal induced level of transport 2.5-fold. This is the first demonstration of an initiator or promoter mutation in an eukaryote.

STUDIES on the regulation of enzyme synthesis in the fungus *Aspergillus nidulans* are increasingly uncovering instances in which such synthesis is subject to multiple forms of regulation. For example, in the accompanying paper¹, it is shown that the synthesis of enzymes involved in proline degradation is inducible and is also repressible by both carbon catabolite and nitrogen metabolite repression. Another example is provided by the *hxB* gene, which codes for a subunit common to two enzymes², each of which is subject both to a specific induction system and to nitrogen metabolite repression³⁻⁵. It is reasonable to assume that adjacent to each multiply regulated structural gene there are receptor sites specific for the respective proteins coded by each regulatory gene involved. These sites may or may not overlap. Evidence for multiple regulatory sites has been reported in the *lac*, *ara*, and, notably, *gal* operons of *Escherichia coli* (refs 6, 7, 8, and references therein).

In *Aspergillus nidulans*, a permease specific for uric acid and xanthine⁹ is both inducible and ammonium-repressible. Expression of the gene coding for the permease requires the products of two positive control genes: the *uaY* gene, necessary for induction (refs 3, 10, and D. J. Cove, H. N. Arst, and C.

Scazzocchio, unpublished), and the *areA* gene, necessary for the expression of all genes subject to nitrogen metabolite (ammonium) repression⁵. Presumably the structural gene (or its RNA transcript) for the uric acid-xanthine permease is adjacent to two receptor sites, one able to bind the product of the *uaY* gene in the presence of coinducer (uric acid¹⁰) and another able to bind the product of the *areA* gene in the absence of corepressor (presumably ammonium). As a great number of enzymes and permeases are subject to ammonium repression, regions of (presumably) DNA able to bind the *areA* product should be widely distributed throughout the genome. Since activities coded by different genes respond in different ways to almost every allele of the *areA* gene⁵, one can only presume that these recognition sites are not all identical. Moreover, mutations in the *areA* gene can also affect the maximal level of synthesis of any of these enzymes or permeases⁵, suggesting that at least a functional overlap might exist between the receptor site for the *areA* product and a promoter site. In the *lac* operon of *E. coli*, not only is there at least a functional overlap between the promoter site (for binding RNA polymerase) and the binding site for the catabolite activator protein^{11,12}, but most operator constitutive mutations show more or less marked promoter effects¹³. Recent research in the bacteriophage λ has shown a physical overlap of promoter and operator sites^{14,15}.

A general method to identify control regions adjacent to structural genes under *areA* control should be to select *cis*-acting mutations which permit the expression of a given structural gene in the presence of a non functional *areA* allele. But most *areA* mutations result in loss of many, if not all, of the activities subject to ammonium repression. As the genes coding for enzymes catalysing sequential steps in a given pathway are generally not clustered in *Aspergillus*¹⁶, mutations at more than one receptor site would be necessary to

* Present address: Department of Biology, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, UK.

Table 1 *In vivo* responses to xanthine, uric acid, and their analogues

Relevant genotype	Inhibition of hypo-xanthine utilisation by allopurinol at		Sensitivity to Inhibition of green pigment formation by 2-thioxanthine or 2-thiouric acid at		Inhibition of growth of strains also carrying an <i>alX</i> ⁻ mutation by xanthine or uric acid at	
	500 ng ml ⁻¹	20 ng ml ⁻¹	100 µg ml ⁻¹	10 µg ml ⁻¹	1 mg ml ⁻¹	50 µg ml ⁻¹
Wild type	S	R	S	R	S	R
<i>uapA</i> ⁻	R	R	R	R	R	R
<i>uap-100</i>	S	S	S	S	S	S
<i>uap-100 uapA</i> ⁻	R	R	R	R	R	R

S=Sensitive; R=resistant

Five mutations leading to defective transport of uric acid and xanthine (*uapA*⁻) were obtained, after NTG mutagenesis, in a strain of genotype *pabaA-1*, as conferring resistance to 100 µg ml⁻¹ 2-thioxanthine, a xanthine analogue⁹, on hypoxanthine as sole nitrogen source. The use of hypoxanthine discards all mutants lacking xanthine dehydrogenase²⁴, leaving *uapA*⁻ mutants as the major class. One mutation, *uapA-24*, was used for transport and genetical studies. Because of the tight linkage of *uapA*⁻ mutations to *uap-100* and the lack of external markers, it was not feasible to obtain *uap-100 uapA*⁻ double mutants by recombination. Therefore other *uapA*⁻ mutations were selected, using the above method, in a strain carrying *uap-100 (uap-100 pyro A-4, auxotrophic for pyridoxine)*. One mutation, *uapA-37*, was selected for further work and was shown not to complement with *uapA-24* in diploids. *uapA-24* and *uapA-37* are both recessive and tightly linked (< 1 centimorgan) to *uap-100* and are therefore allelic. These mutations have been located²⁵ in linkage group I. Growth tests were made on appropriately supplemented solid minimal medium²⁶ at 37 °C. Inhibition by allopurinol was tested using 100 µg ml⁻¹ hypoxanthine as nitrogen source. Inhibition by 2-thioxanthine and 2-thiouric acid was tested using 3.3 mM NH₄⁺ as nitrogen source. Inhibition by xanthine and uric acid was tested using 10 mM NO₃⁻ as nitrogen source. Allopurinol specifically inhibits xanthine dehydrogenase and thus the utilisation of hypoxanthine as nitrogen source⁸. 2-thiouric acid inhibits the conversion of yellow to green conidial pigment^{9,24,27}. 2-thioxanthine is converted by xanthine dehydrogenase to 2-thiouric acid^{9,24}. Xanthine and uric acid are toxic to strains lacking allantoinase (*alX*⁻) resulting from allantoin accumulation⁹. Allopurinol, 2-thiouric acid, 2-thioxanthine, xanthine and uric acid are all substrates of the *uapA* permease^{3,9}.

obtain a revertant. As even single mutations resulting in the ability to bypass the *areA* product or to accommodate a modified *areA* product should be rare, a requirement for multiple mutational events would make the method impracticable.

Nevertheless, advantage can be taken of the wide variety of phenotypes displayed by *areA* mutations. In particular, one allele, *areA-102* (formerly designated *amdT-102*), was selected as resulting in derepression of acetamidase¹⁷. But, *areA-102* also leads to reduced levels of formamidase^{17,18} and notably to an almost total lack of transport activity for uric acid and xanthine, whilst not affecting the catabolism of these compounds once inside the cell⁵. Thus, a revertant able to grow on uric acid and xanthine as nitrogen sources should result from a single mutational event in a *cis*-acting element controlling the uric acid-xanthine permease. This paper describes one such *cis*-dominant mutation, designated *uap-100*, which results in strong constitutivity of the permease as well as a marked 'up-promoter' effect. (An 'up-promoter' effect is an increase in the maximal level of expression of the adjacent structural gene.) *uap-100* does not, however, confer derepression *vis-à-vis* ammonium. This last characteristic is somewhat surprising in view of the method of selection and will be discussed below.

Selection of *uap-100*

uap-100 was obtained after N-methyl-N'-nitro-N-nitrosoguanidine (NTG) mutagenesis¹⁹ of a (*p*-aminobenzoate-requiring) *areA-102* strain (*pabaA-1 areA-102*). Conidiospores

were plated on medium containing uric acid as sole nitrogen source, and 42 revertants were isolated. Forty-one of these revertants have lost at least one other of the characteristics of the *areA-102* phenotype and presumably contain second-site reversions in the *areA* gene. One revertant has exclusively regained the ability to utilise uric acid and xanthine whilst retaining all other characteristics associated with the *areA-102* mutation. As predicted, it carries a specific suppressor mutation. Selection of *uapA*⁻ mutations is described in the legend to Table 1.

In vivo properties of *uapA*⁻ and *uap-100* mutations

Table 1 compares *uapA*⁻, *uap-100* and wild type strains with regard to sensitivity to a number of purines and purine analogues taken up by way of the *uapA* permease^{3,9}. Whereas *uapA*⁻ mutations confer resistance to these compounds, *uap-100* leads to marked hypersensitivity. *uapA*⁻ mutations are completely epistatic to *uap-100*.

Transport and enzyme studies

2-thiouric acid is a gratuitous inducer of both xanthine dehydrogenase I and urate oxidase in *A. nidulans*²⁰. Tables 2 and 3 show that it also induces a uric acid permease which is missing in strains carrying *uapA-24*. The residual uric acid transport in these strains occurs by means of permeases specified by other genes (H. N. Arst and C. Scazzocchio, unpublished).

Table 2 Induction of uric acid transport activity

Relevant genotype	¹⁴ C/ ³ H			
	2-thiouric acid present in growth medium at (μM)			
	0	2.77	27.7	277
<i>uap</i> ⁺	0.0431	0.2763	0.2065	0.1265
<i>uap</i> -100	0.4200	0.6743	0.5403	0.3399

The method for uptake studies has been described in detail previously²⁸. The ¹⁴C counts monitor uric acid uptake while the ³H counts serve as a measure of protein synthesis and hence growth. In addition to the *uap* genotype specified above, both strains carry *yA-2* (yellow conidial colour), *luA-1* (L-leucine auxotrophy), *hxB-13* (loss of xanthine dehydrogenase), and *uaZ-11* (loss of urate oxidase) but are otherwise isogenic. The use of urate oxidaseless strains allows uptake measurements to be made in the absence of any uric acid catabolism²⁹. But *uaZ*⁻ strains accumulate uric acid and are therefore pseudo-constitutive for uric acid-induced activities unless they also lack xanthine dehydrogenase^{20,30}. Hence it is essential to use xanthine dehydrogenaseless strains to study induction. Mycelia were grown for 21 h at 37 °C on solid (growth) medium²⁶ supplemented with 10 µg l⁻¹ biotin, 5 mM urea, 1 mM 4,5-³H-L-leucine at a final specific activity of 500 mCi mol⁻¹, and 2-thiouric acid at the specified concentrations. Mycelia were incubated for 30 min at 37 °C in uptake medium supplemented with 10 µg l⁻¹ biotin, 5 mM urea, 1 mM L-leucine (unlabelled), and 59.5 µM 2-¹⁴C-uric acid at a final specific activity of 3.36 Ci mol⁻¹.

Table 3 Ammonium repression of uric acid transport activity

Relevant genotype or calculation	None	¹⁴ C/ ³ H		
		Additions to growth medium 27.7 µM 2-thiouric acid	10 mM NH ₄ ⁺	27.7 µM 2-thiouric acid + 10 mM NH ₄ ⁺
<i>uapA</i> -24	0.0201	0.0232	0.0120	0.0162
<i>uap</i> ⁺	0.0436	0.2206	0.0470	0.0542
<i>uap</i> -100	0.3931	0.4645	0.0906	0.1165
Activity ratio	15.9	2.3	2.2	2.1

The experimental method was the same as that in the legend to Table 2, except that the additions to the growth medium were those specified above. All strains carry *yA*-2, *luA*-1, *hx*B-13, and *uaZ*-11 (as in Table 2) in addition to the *uap* genotype given above but are otherwise isogenic. The activity ratio is calculated as (¹⁴C/³H for *uap*-100 - ¹⁴C/³H for *uapA*-24)/(¹⁴C/³H for *uap*⁺ - ¹⁴C/³H for *uapA*-24) for each set of growth conditions. Since the residual uric acid transport activity in *uapA*-24 is probably attributable to permeases other than that specified by the *uapA* gene (H. N. Arst and C. Scazzocchio, unpublished), the activity ratio expresses the ratio of uric acid transport activity by way of the *uapA* permease in the *uap*-100 strain to that in the wild type for any given set of growth conditions.

The decline in uptake as higher concentrations of thiouric acid are used to induce probably reflects inhibition of uric acid uptake by preloaded 2-thiouric acid. Tables 2 and 3 show that strains carrying *uap*-100 are strongly constitutive for uric acid uptake. That the permease responsible for this uptake is the one missing in *uapA*⁻ mutants is shown by the complete epistasis of *uapA*⁻ mutations to *uap*-100 (Table 1). On maximal induction, strains carrying *uap*-100 have 2.5 times the level of uric acid transport found in the induced wild type (Tables 2 and 3). Both induced wild type and induced *uap*-100 show approximately fourfold repressibility by ammonium (Table 3).

Table 4 Induction of urate oxidase by a gratuitous inducer

Concentration of 2-thiouric acid	Urate oxidase specific activity	
	Wild type	<i>uap</i> -100
0	<1	<1
27.7 pM	<1	<1
134 pM	3.0	20.9
277 pM	29.3	40.2
2.77 nM	59.5	49.2
27.7 nM	57.6	56.5
277 nM	59.3	50.1

Both strains carry a biotin auxotrophy (*biA*-1) and are, apart from the specified genotypes, isogenic. Mycelium was grown for 20 h at 25 °C in shaken liquid minimal medium²⁸ supplemented with 10 µg l⁻¹ biotin and containing 5 mM urea. 2-thiouric acid was added after 15 h growth. Preparation of cell-free extracts and urate oxidase (EC 1.7.3.3) determinations have been described previously²⁹. Specific activities are defined as nM substrate transformed per min × mg soluble protein. Soluble protein in extracts was determined by the Biuret method³¹.

Data in Table 4 show that the enhancement of uric acid uptake activity by *uap*-100 is reflected in enhanced sensitivity of urate oxidase induction to 2-thiouric acid. The difference is most striking at 134 pM 2-thiouric acid where the *uap*-100 strain is induced to 37% of its maximal level while the wild type is induced to only 5% of its maximal level. Comparable

results were also obtained for xanthine dehydrogenase I (data not shown).

Data in Tables 4 and 5 show that uninduced and induced levels of xanthine dehydrogenase I and urate oxidase are not affected by the *uap*-100 mutation. These enzymes are, as is the *uapA* permease, under the positive control of the *uaY* gene (refs 3, 10, and D. J. Cove, H. N. Arst and C. Scazzocchio, unpublished). Data in Table 5 also show that the apparent degree of ammonium-repressibility of the two enzymes is reduced in *uap*-100 strains when uric acid is used as the inducer. As *uap*-100 has no such effect when hypoxanthine is used to induce, part of the apparent ammonium-repressibility can be attributed to inducer exclusion. Hypoxanthine, which enters the cell by another permease⁹, is able to induce only because it is converted intracellularly to uric acid²⁰.

Cis-dominance of *uap*-100

Table 6 shows the apparent dominance of *uap*-100 in diploids homozygous for *areA*-102. In this case, the *in vivo* test does not distinguish between semidominance and complete dominance. In any event, Table 6 shows clearly that *uap*-100 only affects the expression of a *uapA* gene when located *cis* to it. The presence of *uap*-100 in *uap*-100 *uapA*-37 *areA*-102 strains was verified by crossing to an *areA*-102 strain and recovering progeny able to grow on uric acid as nitrogen source (genotype of progeny *uap*-100 *areA*-102). The presence of *uapA*-37 or *uapA*-24 in strains also carrying *areA*-102 was confirmed by outcrossing to wild type and recovering approximately 25% *uapA*⁻ *areA*⁺ progeny.

Nature of the *uap*-100 mutation

We have not yet established that *uapA* is the structural gene for the uric acid-xanthine permease. The selection of mutants with altered substrate specificity, now in progress, might provide the necessary proof.

Three hypotheses could account for the properties of *uap*-100: (1) The 'up-promoter' effect of *uap*-100 might reflect an altered structure of the permease, increasing the efficiency of uptake. The strong constitutivity of *uap*-100 virtually eliminates this

Table 5 Inducer exclusion by ammonium

Addition at 15 h or calculation	Specific activities			
	Xanthine dehydrogenase Wild type	Xanthine dehydrogenase <i>uap</i> -100	Urate oxidase Wild type	Urate oxidase <i>uap</i> -100
None	10.8	5.8	1.7	1.8
Hypoxanthine	43.9	33.3	35.7	24.1
Hypoxanthine + ammonium	20.6	15.8	3.8	3.0
% Repression with hypoxanthine as inducer	53.1	52.5	95.8	87.6
Uric acid	55.0	41.6	29.8	17.1
Uric acid + ammonium	21.1	27.7	1.5	8.1
% Repression with uric acid as inducer	61.7	33.4	94.1	72.8

Experimental details are given in the legend to Table 4 with the following modifications and additions: 50 µg ml⁻¹ hypoxanthine or uric acid with or without 10 mM ammonium (as the (+)-tartrate) was added after 15 h growth. Xanthine dehydrogenase I (EC 1.2.1.37) was determined according to Scazzocchio *et al.*³. % Repression = (1 - (ammonium-repressed specific activity)/(non-repressed (induced) specific activity)) × 100.

Table 6 *Cis*-dominance of *uap*-100

Relevant genotype	Utilisation of (as nitrogen source)		
	Hypoxanthine	Xanthine	Uric acid
Wild type	+	+	+
<i>areA</i> -102	+	—	—
<i>uap</i> -100 <i>areA</i> -102	+	+	+
<i>uap</i> -100 <i>areA</i> -102	+	+	+
<i>uap</i> -100 <i>uapA</i> -37 <i>areA</i> -102	+	—	—
<i>uap</i> -100 <i>uapA</i> -24 <i>areA</i> -102	+	+	+

Two independent diploids were constructed for both *cis* and *trans* configuration dominance tests. Growth tests were carried out at 37 °C on solid minimal medium²⁶ with purines added at 100 µg ml⁻¹.

possibility. (2) *uap*-100 is a constitutive allele and *uapA*⁻ mutations are noninducible alleles in a positive control gene. This is extremely unlikely as *uap*-100 and *uapA*⁻ mutations do not affect the regulation of the enzymes induced together with the uric acid permease. Moreover, the strong 'up-promoter' effect would be unprecedented and difficult to accommodate to this model. (3) *uap*-100 is a mutation in a control region adjacent to a structural gene, *uapA*, coding for the uric acid-xanthine permease. This hypothesis is consistent with all the data and is definitely the most attractive.

Since the *uapA* gene is under the control of two positive regulatory genes, *uaY* and *areA*, *uap*-100 can be described as an initiator constitutive mutation. Work is in progress to establish the nature of the interaction between *uap*-100 and the *uaY* and *areA* products. Preliminary *in vivo* evidence indicates that *uap*-100 partially suppresses *uaY*⁻ mutations for permeation but does not alleviate the stringent requirement for the *areA* product. This latter finding is in essential agreement with the lack of effect of *uap*-100 on the ammonium-repressibility of the *uapA* permease. Therefore the suppression of *areA*-102 by *uap*-100 implies that *uap*-100, in contrast to its wild type allele, can accommodate to the modified *areA* product present in *areA*-102 strains. Probably the 'up-promoter' effect of *uap*-100 contributes to the magnitude of the suppression. 'Up-' and 'down-promoter' effects are a feature of many initiator constitutive mutations in the *ara* (ref. 21) and *mal* (ref. 22) systems of *E. coli*.

In addition to the *uapA* permease, *areA*-102 results in the loss of at least one other permease for uric acid and xanthine (H. N. Arst and C. Scazzocchio, unpublished). Although *areA*-102 leads to somewhat reduced levels of a number of enzymes and permeases^{5, 17, 18, 23}, these two (or more) permeases with overlapping specificities are the only activities which seem to be totally absent in *areA*-102 strains. This may be the first direct clue that similarity of binding sites for the *areA* product constitutes a physiologically significant form of control. The possibility that *areA* binding sites might exist in discrete classes related to each other by changes involving only a few bases is

suggested by the observation (H. N. Arst and C. Scazzocchio, unpublished) that intracistronic reversions in an *areA*-102 strain yield new *areA* alleles with a phenotype mirroring that of *areA*-102. They result in recovery of uric acid and xanthine uptake activities but in loss of acetamidase.

Pairs of *areA* alleles with antisymmetrical properties and their respective initiator specific suppressors could well provide the means to identify and classify initiator sites in an eukaryote by purely genetical means.

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Determinant of cistron specificity in bacterial ribosomes

J. Shine & L. Dalgarno

Department of Biochemistry, School of General Studies, Australian National University, Canberra ACT 2600, Australia

The sequence of the 3'-terminus of 16S RNA from different bacteria has been determined. Complementarity relationships between this sequence and a purine-rich tract in the ribosome binding site of different bacterial mRNAs suggest that the 3'-end of 16S RNA determines the intrinsic capacity of ribosomes to translate a particular cistron.

INITIATION of protein synthesis in bacteria involves the specific binding of the small ribosome subunit to a region of the mRNA containing the initiation codon. As the initiating 30S subunit discriminates against the many internal AUG or GUG codons and selects only the AUG or GUG triplet at the beginning of a cistron, some feature of the mRNA other than the presence of AUG must be necessary for ribosome recogni-

Table 1 Determination of the 3'-sequence of *C. crescentus* 16S RNA

Number of stepwise degradations before labelling with ³ H-isoniazid	Radioactivity in nucleoside hydrazones released by pancreatic ribonuclease*				Sequence
	G-iNzd	U-iNzd	A-iNzd	C-iNzd	
0	3.5	83.8	8.1	4.6	PyU _{OH}
1	3.5	8.7	8.0	79.9	PyCU _{OH}
2	5.6	74.3	6.4	13.7	PyUCU _{OH}
3	8.3	70.4	9.9	11.3	PyUUCU _{OH}
4	6.0	71.8	5.1	17.1	PyUUUCU _{OH}
5	6.4	19.2	7.3	67.1	PyCUUUCU _{OH}
6	7.6	13.3	8.0	71.1	PyCCUUUCU _{OH}
7	8.6	62.9	11.4	17.1	PyUCCUUUCU _{OH}

C. crescentus (ATCC 15252) was grown and collected as described by Leffler and Szer². RNA was extracted directly from the cell pellet with a mixture of phenol/cresol-aminosalicylate³⁵. DNA, low molecular weight RNA and polysaccharide were removed by washing with 3 M sodium acetate³⁵. 16S RNA was isolated on 5–20% (w/v) sucrose gradients. After 3'-terminal labelling, 16S RNA was digested with pancreatic ribonuclease (100 µg per mg RNA) at 20°C for 4 h in 0.01 M Na/K phosphate buffer (pH 7.4). A sample of the digest (10 or 20 µl) was mixed with 20 µl of unlabelled mononucleoside hydrazones³⁹ and electrophoresed on Whatman 3MM paper at 40 V cm⁻¹ for 2 h in 0.1 M sodium formate (pH 3).

*Expressed as a percentage of total radioactivity in nucleoside hydrazones separated by paper electrophoresis.

tion. Such recognition probably involves some as yet undefined component or feature of the untranslated sequence on the 5'-side of the initiator codon. After the specific interaction of mRNA with the 30S subunit, stabilisation of the initiation complex is dependent on initiation factors¹⁻³, and possibly on the 'fractional' ribosomal protein S₁ (ref. 4).

Cistron specificity

A number of reports demonstrate that ribosomes from different bacterial species show cistron specificity in the translation of natural mRNAs *in vitro*, irrespective of the source of the initiation factors used^{2,5-11}. Thus, ribosomes from *Micrococcus cryophilus* or *Pseudomonas* translate protein from all three cistrons of MS2 RNA in the same relative amounts as those from *Escherichia coli*⁸. Under comparable conditions *Bacillus stearothermophilus* ribosomes translate only the A-protein cistron of R17 or f2 RNA (refs 5–7). *Caulobacter crescentus* ribosomes are unable to translate any of the MS2 RNA cistrons²; correspondingly, *E. coli* ribosomes do not translate RNA from the *C. crescentus* phage Cb5 (ref. 2). A similar cistron selectivity is also demonstrated, in the absence of initiation factors, in a weak, but species-specific interaction of ribosomes with certain phage RNAs (ref. 4).

Studies with hybrid ribosomes show that the specificity of ribosome binding and translation of natural mRNAs is primarily a function of the 30S subunit^{2,7}. Two recent reports aimed at determining the component of the 30S subunit responsible, argue that both a ribosomal protein (particularly protein S12) and the 16S RNA determine the specificity of initiation^{12,13}. The type of recognition process involved, however, is unknown.

Base sequence or secondary structure?

The recognition of mRNA initiation signals by 30S subunits could conceivably be determined by a specific base sequence on the 5'-side of the initiation triplet, by the degree or type of secondary structure in this region of the mRNA, or by both factors. Ribonuclease digestion of initiation complexes formed on specific mRNAs has made it possible to determine the base sequence of ribosome-binding sites for several mRNA cistrons^{6,14-23}. Although some of these ribosome-protected sequences probably have a fairly high degree of secondary structure^{6,14,19,24}, no such secondary structure seems possible for other initiation sites^{23,25,26}. It therefore seems unlikely that the 30S subunit specifically recognises some feature of the secondary structure of the ribosome-binding site sequence, particularly since a reduction in structure on partial denaturation of intact mRNAs generally increases, rather than decreases, the number of available initiating sites^{27,28}. This observation therefore suggests that the specific binding of

ribosomes to mRNA may depend on the ribosome-binding site being in an open, single-stranded form, rather than in a structured conformation.

It is significant therefore that all coliphage RNA ribosome-binding sites examined to date contain all or part of the purine-rich sequence 5'-AGGAGGU-3' in a similar relative position on the 5'-side of the initiator triplet AUG. The ribosome binding site of an endogenous *E. coli* mRNA also contains part of this sequence, AGGA (ref. 23). We have previously shown that the 3'-terminus of *E. coli* 16S RNA contains the sequence 5'-ACCUCCU-3' which is the complement of AGGAGGU (ref. 29). This, together with evidence that an intact 3'-terminus of 16S RNA is necessary for protein synthesis in bacteria³⁰⁻³² and more recent data suggesting a specific role for the 3'-terminus in initiation³³, have prompted us to suggest that the 3'-terminal sequence of 16S RNA may have a direct base-pairing role in the initiation of protein synthesis on natural mRNAs (ref. 29).

This hypothesis predicts that there will be a positive correlation between the translation of a particular bacterial mRNA cistron by bacterial ribosomes, and the degree of complementarity which exists between the ribosome binding site sequence of that cistron and the 3'-terminal sequence of the 16S ribosomal RNA. We have therefore determined the 3'-terminal sequences of 16S RNA from *P. aeruginosa*, *B. stearothermophilus* and *C. crescentus* since information exists on both the capacity of ribosomes from these bacteria to translate various bacterial mRNA cistrons, and on the ribosome binding site sequences of the cistrons concerned.

3'-terminal sequences

A procedure for the stepwise removal of seven to eight nucleotides from the 3'-terminus was applied to 16S RNA isolated from bacteria (see Tables 1 and 2). This is a cyclic process and involved removal of the periodate-oxidised 3'-nucleoside by incubation in 0.33 M aniline, digestion with alkaline phosphatase to release the 3'-phosphate and periodate-oxidation of the resultant 2',3'-hydroxyl groups^{29,34}. After removal of successive 3'-nucleosides, the RNA was labelled by condensation with ³H-isoniazid³⁵ and digested with specific nucleases. The labelled 3'-terminus was characterised by chromatography of the ribonuclease digest on DEAE-Sephadex and identification of any 3'-nucleoside hydrazones by paper electrophoresis^{36,37}.

An outline of the sequence determination for *C. crescentus* 16S RNA is shown in Table 1. The 3'-sequences of 16S RNA from the other bacteria, determined by the same method, are listed in Table 2. A general feature of all the 3'-termini is the high proportion of pyrimidine residues. Apart from the terminal adenosine, the seven terminal nucleotides at least, are invariably uridylic acid or cytidylic acid.

Complementarity to coliphage ribosome-binding site sequences

We have previously shown that the 3'-terminal nucleotides of *E. coli* 16S RNA could form seven, four and three base pairs with the appropriate region of the R17 A-protein, replicase and coat-protein ribosome-binding sites, respectively (see Table 3 and ref. 29). The extent of this pairing relates closely to the ribosome-binding capacities of the three isolated initiator regions of R17 RNA since *E. coli* ribosomes discriminate in favour of the A-protein initiator fragment some fortyfold and elevenfold over the coat and replicase sites, respectively³⁸. This is in contrast to initiation of the three cistrons on intact R17 RNA where some 20 moles of coat protein and 5 moles of replicase are synthesised for each mole of A-protein⁴⁴. Under these conditions the secondary structure of the intact phage RNA presumably impedes binding of the 30S subunit to the A-protein and replicase initiator regions³⁸.

The 3'-end of *Ps. aeruginosa* 16S RNA is identical to that of *E. coli* for seven nucleotides; it can be aligned such that five, four and three to four base pairs are possible with the ribosome-binding site sequences of the A-protein, replicase and coat-protein cistrons of R17 RNA, respectively (Table 3). It has been previously shown⁸ that *Pseudomonas* ribosomes translate all three cistrons of MS2 RNA in a manner analogous to that of *E. coli* ribosomes.

B. stearothermophilus (and *B. subtilis*) 16S RNA has a 3'-sequence significantly different to that of *E. coli* and *Ps. aeruginosa* 16S RNA (Table 2). Some complementarity ($\geq$ four base pairs) exists, however, between this sequence and the ribosome-binding sites of both the A-protein and replicase cistrons of R17 RNA (Table 3). The degree of complementarity possible with the coat-protein binding site is considerably less (one to two base pairs). *B. stearothermophilus* ribosomes bind significantly only to the A-protein site on native f2 or R17 RNA (refs 6 and 7). Appreciable translation by *B. stearothermophilus* ribosomes of both the replicase and A-protein cistrons does occur, however, after unfolding of the RNA with formaldehyde²⁸; under these conditions no coat protein is synthesised. *B. stearothermophilus* ribosomes also exhibit a relatively high level of recognition of the R17 replicase initiation site when assayed at 49 °C (ref. 13); the T_m of the short helix containing the R17 replicase initiator region is 48 °C in 0.05 M Na⁺ (ref. 24). *B. stearothermophilus* ribosomes are therefore intrinsically able to recognise the A-protein and replicase sites but not the coat-protein site, as would be predicted if a minimum of three to four base pairs were necessary to permit stable interaction. Presumably the conformation of the native, intact phage RNA usually renders the replicase site inaccessible to *B. stearothermophilus* ribosomes.

B. stearothermophilus ribosomes bind to only a single site on Q β RNA at 65 °C, which does not correspond to any of

the three normal initiator regions²⁸. The ribonuclease-protected fragment is similar in size to that bound by ribosomes during productive initiation on the A-protein cistron of R17 RNA. It contains no initiation triplet, showing that mRNA can be specifically recognised and bound by ribosomes independently of polypeptide chain initiation²⁶. The fragment, however, contains a polypurine tract of composition G(AAAG,AG,G,G,G)A in a similar relative position to that of AGGAGGU in the R17 A-protein site²⁶. It is significant therefore that the complement of the 3'-terminus of *B. stearothermophilus* 16S RNA is AGAAAGGA.

As the 3'-sequence of *C. crescentus* 16S RNA is similar to that of *B. stearothermophilus* 16S RNA (Table 2), one could predict a similar recognition by *C. crescentus* ribosomes of both the A-protein and replicase cistrons of R17 RNA (Table 3). This prediction is not inconsistent with the observed failure of *Caulobacter* ribosomes to translate MS2 RNA at 37 °C, as under these conditions the initiation sites for the A-protein and replicase cistrons are presumably largely masked by the secondary structure of the phage RNA (refs 24 and 38). This is analogous to the very low level of translation of f2 RNA by *B. stearothermophilus* ribosomes at 37 °C compared to that found at 65 °C or after mild denaturation of the RNA with formaldehyde²⁸. It is significant that considerable translation of formaldehyde-treated MS2 RNA occurs with *Caulobacter* ribosomes², although the identity of the translated cistrons is not known.

The degree of possible interaction between the 3'-terminus of 16S RNA from several different bacteria and the ribosome-binding sites of coliphage RNA is therefore consistent with the available data on translational specificity in these bacteria. Confirmation of the hypothesis that the 3'-end of 16S RNA is directly involved in the selection of initiation regions on mRNA must await the sequencing of ribosome-binding sites from other bacterial messengers and direct demonstration of such an interaction. For instance, we would predict that the sequences of the ribosome-binding sites from RNA of the *C. crescentus* phage Cb5 would contain some part of the sequence complementary to the 3'-end of *C. crescentus* 16S RNA, that is, some part of AGAAAGGA.

Initiation factors

The role of initiation factors (for example, IF-3) in determining the efficiency of initiation on certain cistrons or mRNAs seems to be related to their capacity to stabilise the mRNA-30S subunit complex¹⁻³. 30S subunits, possibly through the 3'-end of 16S RNA and protein S12 (ref. 12), have an inherent capacity to bind weakly but specifically to mRNA (ref. 4) and are stabilised in this interaction by various initiation factors, particularly IF-3 (refs 1-3) and the ribosomal protein S₁ (ref. 4).

The role of IF-3, which interacts with both 30S subunits and with mRNA (refs 1-3, 45), could be to potentiate or stabilise base pairing between the 3'-terminus of 16S RNA and the complementary region of the ribosome-binding site. Purified IF-3 species promote differential translation of coliphage RNA cistrons⁴⁶⁻⁴⁸; this may reflect a greater effect of IF-3 in the stabilisation of the mRNA-30S subunit complex at particular initiation sites. Thus initiation of the coat-protein cistron of R17 RNA, where only three to four base pairs can be formed between mRNA and the 3'-end of *E. coli* 16S RNA, may be highly dependent on the stabilising presence of IF-3 compared to initiation at the A-protein cistron, where seven such base pairs are possible. Such a view is consistent with the effect of IF-3 on ribosome binding to unfolded MS2 RNA, where addition of IF-3 stimulates ribosome binding to the coat-protein site but not to the other sites⁴⁸. Similarly, crude initiation factors produce a significant stimulation in the binding of high-salt-washed ribosomes to the R17 initiator regions. This effect is about elevenfold, eightfold and fourfold for the coat-protein, replicase and A-protein sites, respectively²⁶.

Table 2 3'-terminal sequences of 16S rRNA

<i>E. coli</i> B	GAUACACCUCCUUA _{OH} (ref. 29)
<i>P. aeruginosa</i>	G(X) ₂ PyCUCUCCU(A) _{OH} *
<i>B. stearothermophilus</i>	G(X) ₂ PyUCCUUCU(A) _{OH} *
<i>B. subtilis</i>	G(X) ₂ PyCUUUCU _{OH}
<i>C. crescentus</i> (ATCC 15252)	G(X) ₃ PyUCCUUCU _{OH}

Bacteria in mid-log phase were converted to protoplasts and extracted with a solution of phenol/cresol-aminosalicylate (see ref. 29 and legend to Table 1). For all bacterial species examined, chromatography on DEAE-Sephadex of T₁-ribonuclease digests of terminally-labelled 16S RNA demonstrated the presence of a large ($\geq$ 12 nucleoside residues) 3'-terminal T₁-oligonucleotide. X represents any nucleoside other than guanosine. A more detailed account of the sequences and their determination is in preparation.

*The variable presence of the 3'-terminal adenosine in 16S RNA is found in a variety of bacteria and depends on the culture conditions. A more extensive examination of this phenomenon will be the subject of a separate report.

Table 3 R17 ribosome-binding site sequences^a and proposed pairing with the 3'-terminus of 16S RNA

Bacteria	R17 cistron	Possible pairing of 3'-terminus with appropriate region of ribosome-binding site sequence*	Number of base-pairs possible	Ribosome binding to unfolded R17, MS2 or f2 RNA (refs 2, 6, 8, 27, 28, 38)
<i>E. coli</i> B	OH AUUCCUCCAC Py	16S RNA (ref. 29)		
	A-protein	(5') CU <u>AGGAGGU</u> UU(3')	7	+
	Replicase	A CAU <u>GAGGA</u> AUU	4	+
	Coat protein	A CC <u>GGGG</u> GUUUG	3(4)††	+
		↓ A †		
<i>Ps. aeruginosa</i>	OH AUUCCUCUC Py	16S RNA		
	A-protein	(5') CU <u>AGGAGGU</u> UU(3')	5	+
	Replicase	UG <u>AGGA</u> UUA C	4	+
	Coat protein	A CC <u>GGGG</u> GUUU	3(4)	+
		↓ A †		
<i>B. stearothermophilus</i>	OH AUCUUUCCU Py	16S RNA		
	A-protein	(5') AUCCU <u>AGGAG</u> G(3')	≥4	+
	Replicase	A CAUG <u>AGGA</u> U	4	+
	Coat protein	A ACCGG <u>GGU</u> U	2(1)	—
		↓ A †		
<i>C. crescentus</i>	OH UCUUUCCU Py	16S RNA		
	A-protein	(5') UCCU <u>AGGA</u> G(3')	≥4	See text
	Replicase	CAUG <u>AGGA</u> U	4	and ref. 2
	Coat protein	A CCGG <u>GGU</u> U	2(1)	
		↓ A †		

*The sequence given represents that containing all or part of the conserved sequence 5'-AGGAGGU-3' from the A-protein, replicase and coat-protein initiator regions of R17 RNA. The initiator AUG is located eight to nine bases to the 3'-side of this sequence. Apart from the G→A transition (see below), these sequences are identical to those available for the corresponding regions of f2 (ref. 15) and MS2 RNA (refs 18, 21, 22 and 40).

†Two sequences have been reported for this region of the coat-protein binding site in the two different R17 stocks used^{6,41}; the G→A transition probably represents a spontaneous mutation which occurred after the two stocks were separated⁴¹. Such a transition may have some selective advantage since it would increase the stability of interaction between the coat protein initiation site and the 3'-end of 16S RNA. The corresponding sequence from MS2 (refs 22 and 40) and f2 RNA (ref. 15) contains the A substitution. The *in vitro* ribosome-binding data were obtained with R17 RNA containing the GGGG sequence^{6,38} and with f2 or MS2 RNA presumably containing the GGAG sequence^{2,8,27,28}.

††The apposition of a G and a U residue in the proposed helical region formed between the coat-protein binding site and 16S RNA would cause little, if any, destabilisation of the base-paired structure^{42,43}.

The bacterial protein, factor *i* (interference factor), also modifies cistron selection in the translation of coliphage messengers by bacterial ribosomes. Significantly, it seems to be identical to the fractional 30S ribosomal protein S₁ (ref. 49), which can be linked chemically to the 3'-terminus of 16S RNA *in situ*⁵⁰ and which is necessary for the specific, initiation factor-independent binding of 30S subunits to mRNA (ref. 4).

Factor *i* is also identical to the α-subunit of the Qβ replicase complex⁵¹; the presence of the α subunit is essential for the binding of the replicase to the 3'-terminus of the Qβ+ strand⁵². The 3'-sequence of this RNA is UCUCUCCCA_{OH} (ref. 53), and the 3'-sequence of 16S RNA is UCACCUCCUUA_{OH}, that is, they both share the sequence CCUCC which we propose to be involved in interaction with ribosome-binding sites. As factor *i* has a specific affinity for certain polypyrimidine tracts in RNA (ref. 54), its complex regulatory function may relate to a capacity for recognising such nucleotide sequences in mRNA and at the 3'-terminus of 16S RNA.

The inhibitory activity of factor *i* on the translation of

coliphage RNA is restricted to initiation at the coat protein cistron⁵⁵ and is a function of mRNA concentration⁵⁴⁻⁵⁶. This effect may be the result of both a direct binding of factor *i* to a region of the mRNA close to the coat protein initiation site⁵⁶, particularly at high concentrations of factor *i*, or to a competition between the factor and mRNA for a ribosomal site⁵⁴, possibly the pyrimidine-rich 3'-end of 16S RNA. It has recently been proposed that factor *i* bound to the ribosome may be directly displaced by added mRNA (ref. 54), the efficiency of which may depend on the sequence of nucleotides on the mRNA to which the ribosomes bind. Thus initiation at the R17 coat-protein binding site, which can form only three to four base pairs with the 3'-terminus of 16S RNA, is inhibited by the presence of factor *i* (refs 54-56), whereas initiation at the A-protein site (7 possible base pairs) is unaffected⁵⁵.

The ribosomal protein S₁₂ is also clearly involved in specific initiation on mRNA (ref. 12). However, its role may result from its ability to interact with initiation factors on the 30S subunit¹³, perhaps potentiating the specific recognition by the 3'-end of

16S RNA. Using bifunctional cross-linking reagents it has recently been shown (Heimark, R., and Traut, R. T., and Bollen, A., Kahan, L., Cozzone, A., Hershey, J. W. B. and Traut, R. R., quoted in ref. 49.) that S_1 (factor i), S_{12} and IF-3 give cross-linked products in the 30S subunit, and are presumably close to the 3'-end of 16S RNA, since S_1 can be chemically linked to the 3'-terminal nucleoside⁵⁰.

We therefore propose that the cistron specificity of bacterial ribosomes previously ascribed to the 30S subunit, can be further localised to a pyrimidine-rich stretch of about ten nucleotides at the 3'-terminus of the 16S ribosomal RNA. We suggest that the degree of complementarity of this region with a purine-rich segment within the ribosome binding site sequence on bacterial mRNA determines the intrinsic capacity of the ribosome to translate a particular cistron. The finer controls imposed on initiation at different cistrons may involve both the accessibility of the purine-rich segment to the 3'-terminus as determined by the secondary structure of the initiation region, and the availability of certain initiation factors which would modify the proposed interaction.

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letters to nature

Submillimetre brightness spike at the solar limb

WE report here the first complete phase of the reduction of data obtained during the solar eclipse of June 30, 1973 from the high altitude moving platform provided by Concorde 001. Reports of the flight¹ and of an optical measurement of chromospheric thickness² are already published. Submillimetre observations were made at both the second and third contacts, using a rapid-scanning Michelson interferometer. At the second contact one complete interferogram of resolution 1 cm⁻¹ was measured each second, but the limited passband of the detector, an InSb Rollin instrument³, was found to have attenuated the highest frequencies (above 200 Hz) so these data have not been used. At the third contact 10 s were taken per interferogram. The instrument response was satisfactory, but the record needed subsequent digitisation, rendering analysis much lengthier, since an electronic analogue Fourier transformer had been built for 1 s interferograms, but was not usable for the 10 s data.

Figure 1 shows a plot of solar flux against the lunar limb position in 3 passbands, centred at 400 μ m, 800 μ m, and 1,200 μ m. Radiation from the image of the revealed solar crescent, plus that from the lunar disk, and from the sky field

was plotted against time in each band. Time was then correlated with the limb positions using aircraft coordinates and astronomical data. The lunar and sky contributions were then subtracted, leaving the solar contribution as a function of the distance of the lunar limb from the solar optical limb. Corrections for several effects were taken into account. First, for atmospheric emission and absorption, the latter averaging less than 10% in the shortest, most obscured waveband. Second, for spectrally selective absorption and emission by the 12 mm thick, crystal quartz, window of the aircraft. Third, for spectrally selective absorption by the TPX lenses in the spectrometer and the TPX detector window. Finally, for the wide lobes of the 12-cm telescope, which had 30' diffraction limited resolution at 1,200 μ m. The sky brightness temperature above 1,200 μ m averaged 15 K above 57,000 feet, giving an accuracy limit of 1% of the total signal, which limited the relative radiometry.

The separate contributions from the whole lunar disk and from the sky were measured with considerable precision during the approximately 65 min of submillimetre totality, by integrating alternately on and off the image of the lunar disk. During the 7 min observing time starting at the origin of Fig. 1 the power on to the detector from the Moon, within the instrument passband, was 3×10^{-8} W; from the sky it averaged 1.5×10^{-8} W. The power from the solar crescent when the optical

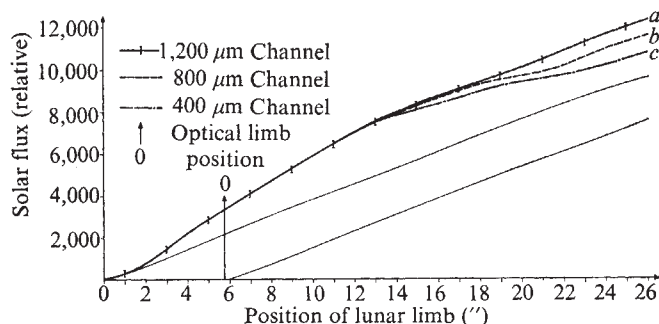


Fig. 1 Flux from the Sun against the lunar limb position in 3 submillimetre wavelength bands. Error bars shown on the 1,200 μm curve apply closely to the other wavelengths. Two lower plots show computed fluxes from theoretical models with no limb brightening. Arrow indicates the position of the optical limb.

limb was just revealed, at point 0, was estimated to be 1.5×10^{-9} W. The minimum detectable signal, expressed as the noise equivalent power resulting from the combined detector noise and the local emission noise was 8×10^{-12} W Hz $^{-1}$. Thus, integration times of the order of seconds, and the dynamic range of 10^3 to 1, yielded good signal-to-noise ratios in each of the 3 wavelength channels. Nevertheless, a multichannel radiometer would have given superior values in this non-detector noise limited broad-band regime.

Also shown in Fig. 1 are computed curves for solar disks of a constant brightness temperature of 5,750 K, together with radii equal to the optical radius and 6'' larger, respectively. These show that there is an excess at the limb both geometrically and thermally distinguishable from 'flat-topped' distributions. This is displayed clearly in Fig. 2, which assumes circular symmetry about the centre of the solar disk. The plots have a resolution, obtained by smoothing curves with an original 0.8'' discrimination, of 1.6''. That is superior by a factor of 25 to the resolution obtained, at 1,400 μm only, with the largest ground based millimetre-telescope⁴; and by a factor of 50 to the resolution obtained in the two submillimetre wavebands⁵. The brightness spike at the limb has been normalised to fit a brightness temperature, T_b at the centre of the disk, of 5,750 K in the 1,200 μm band. A good fit to the

observations was then obtained by assuming the peak of the spike at 800 μm and 400 μm to have the same T_b value (10,100 K) as at 1,200 μm . That leads to extrapolated disk temperatures at 800 μm and 400 μm of 5,200 K and 4,700 K, respectively, which are in reasonable agreement with previous measurements^{6,7}. As only 19'' of photospheric disk were observable, however, these extrapolations rely heavily on a model which assumes that the Sun is a diffuse emitter except for the source responsible for the observed spike.

The very limited angular extent of the excess emission, which extends from some 4'' outside the optical limb, where it exhibits a very steep cutoff, to 5'' within the optical limb, with a less steep decline, suggests that the spike can be identified with emission from chromospheric spicules. This geometrical interpretation of the excess emission is corroborated by the apparently small change of peak temperature with wavelength. Models of spicules from data at optical wavelengths⁸, imply that each spicule is optically thick throughout the submillimetre range. Though sparsely distributed, their superposition at the limb is here seen as the edge of a thin sheet at temperatures of the order of 10,000 K. A more detailed analysis should yield information on the number density and length distribution of the spicules above the photosphere, in the region near the chromopause.

Although an occultation experiment of this type is well suited to examining the large scale brightness distributions across the Sun, the limited time that was available after the third contact in this case has prevented such an examination here. The mystery of the submillimetric limb brightening, missing on an arc minute scale^{7,9} thus remains.

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J. E. BECKMAN*
J. C. G. LESURF
J. ROSS

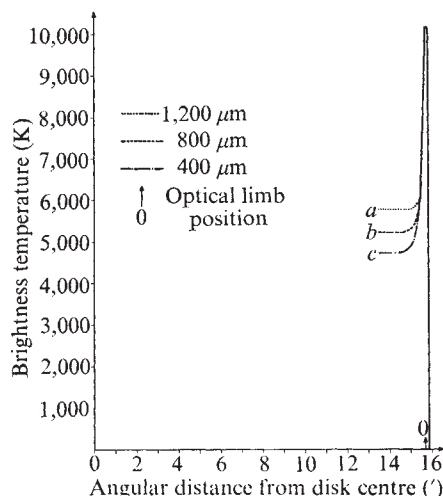
Department of Physics,
Queen Mary College,
London E1 4NS, UK

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*Present address: Astronomy Division, ESTEC, Noordwijk, The Netherlands.

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Fig. 2 Brightness temperature against angular distance from the centre of the solar disk. Dotted curves are partly extrapolated. The solid curve, common to all three channels, is normalised at peak. Arrow indicates the position of the optical limb.



Circular polarisation in HDE226868

MUCH effort is being expended to develop an understanding of the details of X-ray production in some astronomical sources. Accretion on to magnetic poles is the mechanism most often invoked for X-ray production in collapsed stars. Strong magnetic fields, such as those associated with collapsed objects, are capable of producing observable circular polarisation; so it is of great interest to determine whether HDE226868, the binary containing Cyg X-1, exhibits this effect.

Nikulin, Kuvshinov and Severny¹ reported circular polarisation from the light of HDE226868 that varied with time between 1.2% and 2.1%. Subsequently, Gehrels², using a matching

Table 1 Time-averaged circular polarisation values for HDE226868

Filter	n	$(10^4) q \pm \sigma q$
U	2	$+1.7 \pm 4.1$
B	8	$+2.9 \pm 0.9$
V	1	$+4.0 \pm 3.0$
r	3	-5.1 ± 0.9
Infrared	1	-4.6 ± 2.5

colour response, observed no significant circular polarisation down to a level of about 0.05%. Furthermore, he found no polarisation in the visible or ultraviolet regions down to levels of about 0.1%.

Using the photoelastic polarimeter and 31-inch telescope at Battelle Observatory³, we have obtained circular polarisation measurements of Cyg X-1 on nine nights during June–October, 1974 (Table 1). The fourth Stokes parameter V , normalised to unit intensity, is denoted by q , and n is the number of observations for a given filter. Positive circular polarisation refers to counterclockwise rotation of the electric vector in a fixed perpendicular plane for an observer facing the source. The effective wavelengths are 3,700, 4,200, 5,350, 6,450, and 7,250 Å for the U, B, V, r, and infrared filters, respectively. The half-power transmittances are $\lambda\lambda$ 3,450–3,900 Å, $\lambda\lambda$ 3,800–4,750 Å, $\lambda\lambda$ 5,100–5,700 Å, $\lambda\lambda$ 6,000–7,200 Å, and $\lambda\lambda$ 6,750–7,800 Å.

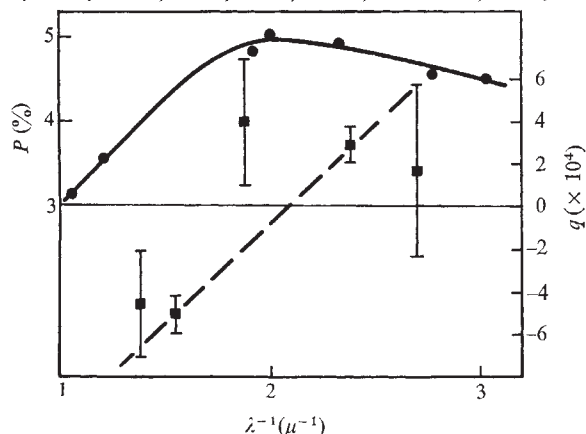


Fig. 1 Linear (●) and circular (■) polarisation of HDE226868 as a function of wavelength. The linear polarisation is taken from Gehrels².

There is definite circular polarisation ($\geq 3\sigma$) in two filters (B, r). The wavelength dependence of the linear polarisation, as presented by Gehrels² and plotted in Fig. 1, suggests interstellar polarisation. Coyne, Gehrels, and Serkowski⁴ predict a maximum linear polarisation at about, 5,000 Å. From calculations based on the analysis of Martin⁵, we expect a circular polarisation near zero at this wavelength, and in the blue and red regions we expect approximately the same magnitude, but opposite handedness for q ; this is roughly what is seen in Table 1 and Fig. 1. Narrow-band measurements of both the linear and circular polarisation over the entire spectrum would help to separate interstellar and any possible intrinsic effects.

The case for interstellar dust as the source of the circular polarisation is strong. But the interest in this object warrants further polarimetric investigations.

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J. J. MICHALSKY
J. B. SWEDLUND

Battelle Pacific Northwest Laboratories,
Richland, Washington 99352

Department of Physics and Astronomy,
University of Kentucky,
Lexington, Kentucky 40506

R. W. AVERY

Received November 26, 1974.

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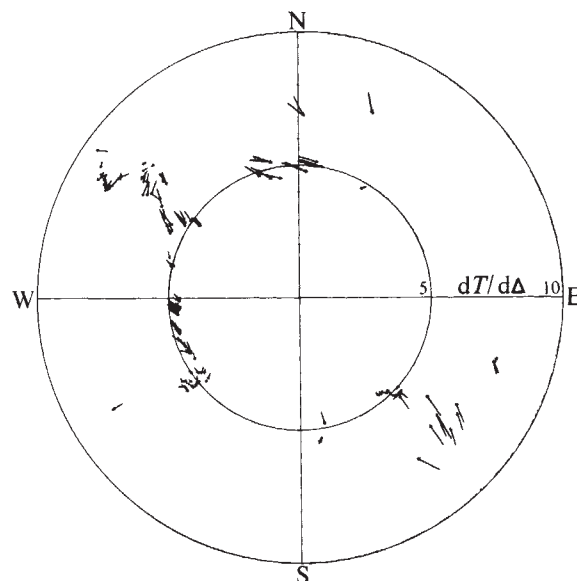
Evidence for mantle heterogeneity from two large seismic arrays

OBSERVATIONS from two large seismic arrays suggest that lateral heterogeneity is present throughout the Earth's mantle. The differences between seismic event locations determined by the arrays and event locations determined by a global network of seismic stations indicate that seismic rays are deflected by velocity anomalies in the mantle.

A large array can be used to locate seismic events because it provides a direct measurement of the azimuth and inclination (or wave slowness $dT/d\Delta$) of incoming teleseismic P-waves. Such locations, however, are usually inaccurate because they are very sensitive to the effects of structure along the propagation path. By contrast, locations determined by a global distribution of seismic stations and reference to standard travel time tables are accurate to within 30 km because the effects of small scale structure in the crust and mantle have been averaged out.

Davies and Sheppard¹ suggest that large errors in event locations determined by the Large Aperture Seismic Array (LASA) must be attributed to lateral heterogeneity in the mantle. If most of the heterogeneity is a long way from LASA, other arrays which sample the same source regions and approximately the same propagation paths should show similar mislocation tendencies. I have determined locations for teleseismic events recorded at a large seismic network situated near Hanford, Washington. The network contains 24 permanent stations, irregularly distributed within an area roughly 100×150 km. All stations consist of 1-s vertical seismometers, modified to record higher frequency microearthquake activity. This precludes detection of teleseismic events with magnitudes below 5.7, so that only about 200 acceptable events were recorded between 1970 and 1972. Two stations were eliminated because their travel time residuals show anomalously high scatter; the remaining 22 stations form the Hanford array. Arrival times were chosen visually at the first coherent peak or

Fig. 1 The Hanford array diagram. The radial axis is $dT/d\Delta$ and the polar axis is array to event azimuth. Each arrow represents a mislocation vector for one event; the head is the true location and the tail is the apparent location. Mean vector 0.38 s per degree along azimuth 245° removed as described in ref. 1.



trough. Reading accuracy is about 0.1 s which limits the accuracy with which events are located to ~ 200 km.

The azimuth and $dT/d\Delta$ of incoming teleseismic signals were determined by a least squares fit of the best plane wave to the uncorrected data. These quantities, together with the azimuth and $dT/d\Delta$ values predicted by the United States Coast and Geodetic Survey (USCGS) "preliminary epicentre" locations and reference to the Jeffreys-Bullen (JB) tables, were plotted on an array diagram (Fig. 1). Seismic source regions can be located using Fig. 2.

A first order correction¹ for the effect of gross structural trends directly beneath the array has been applied to the data of Fig. 1.

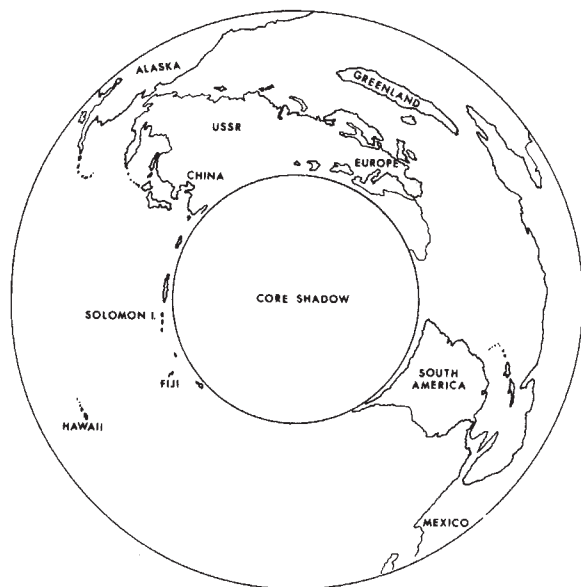


Fig. 2 The coastlines of the world as viewed through an array diagram centred on the Hanford array, assuming that the JB tables for P-phases are correct. This map was created by R. Sheppard

The Hanford array mislocates most seismic events. Systematic mislocations are associated with several source regions such as Russia, the Ryukyu, Bonin and Solomon Islands and the northern portion of South America. Rapid variations in both azimuth and $dT/d\Delta$ anomalies are also present, notably associated with events from the southern end of the Solomon Islands, the coast of Chile and the island of Hokkaido.

An array diagram for LASA is shown in Fig. 3. The coastlines of the world as viewed through an array diagram centred at LASA are very similar to those shown in Fig. 2. LASA also systematically mislocates events from several source regions, and the direction of mislocation is strikingly similar to that observed in the Hanford array diagram. Some of these features are more easily seen in Fig. 4.

The similarities in the global pattern of mislocations for the arrays suggests that structure at considerable distance from the arrays is responsible for most of the mislocation. The only alternative explanation requires nearly identical structure beneath both arrays which is unlikely. My analysis supports the suggestion¹ that most of the mislocation is a result of lateral heterogeneity in the mantle.

Azimuth and $dT/d\Delta$ anomalies associated with several source regions are too large to be attributed to structure associated with descending slabs of lithosphere¹⁻⁴. Most of the orientations of the mislocation vectors show no obvious correlation with the dip of the descending slab or with depth to source. There is a tendency, however, for the orientations of the mislocation vectors to change from one trench region to the next, as shown by the data for the Hebrides and Solomon Islands

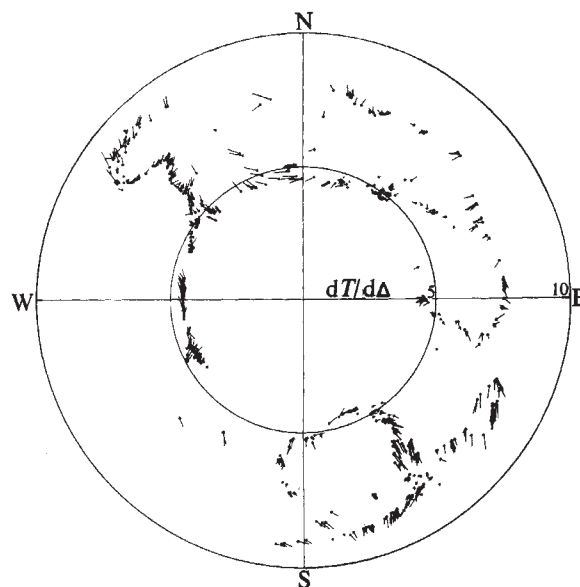


Fig. 3 An array diagram for LASA. Data used to construct this diagram are the same as those used by Davies and Sheppard¹. Mean vector removed as in ref. 1.

(Fig. 4). Similar effects are seen in the LASA data. This suggests that structure close to the source regions must be responsible for at least part of the observed mislocation. As the descending slab cannot be responsible, it seems that we are observing the effects of structure on deeper interfaces such as the 630 and 800 km discontinuities.

Events from Russia seem to be arriving 5° off azimuth at both LASA and Hanford. A significant difference should be observed between the average JB time residuals calculated for each array if the incoming wave fronts were deflected at great distance from the arrays, for instance in the deep mantle, and were not seriously distorted during their subsequent traverse to the Earth's surface. The magnitude of the time

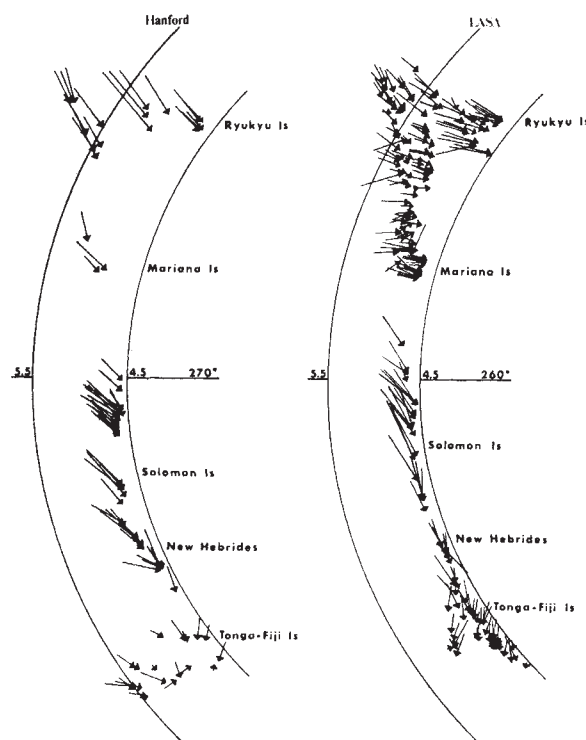


Fig. 4 Enlarged portions of the LASA and Hanford array diagrams.

difference is approximately $t = H \cos \theta \delta \theta / V$ where H is the distance between the arrays, θ is the angle between the line connecting the arrays and the wavefronts (if the wavefronts suffer no distortion) $\delta \theta$ is the azimuth anomaly and V is the apparent velocity of the waves as they sweep across the array. For the Russian events, $H = 900$ km, $\theta = 6^\circ$, $\delta \theta = 5^\circ$ and $V = 22$ km s⁻¹; the expected time difference is about 3.5 s. This has not been observed. Even allowing for dissimilar $dT/d\Delta$ anomalies at each array and for variations associated with travel time data⁵ it is extremely difficult to mask an expected time difference of 3.5 s. This suggests that similar structure in the upper mantle directly beneath both arrays may be responsible for the mislocation of Russian events. But the general agreement in the global pattern of mislocations at both arrays suggests that upper mantle structure beneath the arrays is not responsible for most of the features in the array diagrams.

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CHRISTINE POWELL

Department of Geological and Geophysical Sciences,
Princeton University, Princeton, New Jersey 08540

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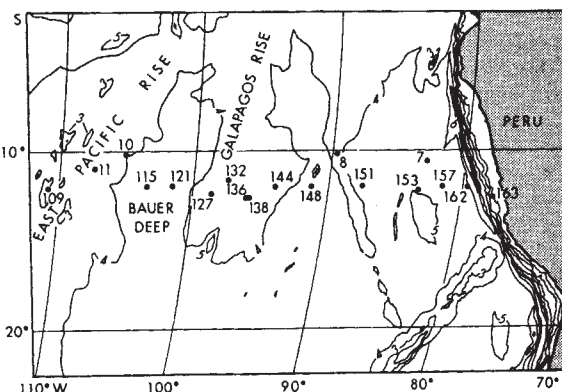


Fig. 1 Index and core location map showing general bathymetry in the 12°S region of the Nazca plate.

in the sediment column⁷. Our geochemical evidence supports the hydrothermal metallogenesis hypothesis.

The Bauer Deep, located between the East Pacific Rise and a fossil spreading centre, the Galapagos Rise⁸, is an area of sediment accumulation rich in metals (Fig. 1). This sediment may originate from East Pacific Rise crest exhalations that are transported into the Bauer Deep⁹. Heat flow values for the Bauer Deep are larger than values for other oceanic basins of the same age¹⁰; the anomalously high heat flow values in the Bauer Deep with their large variability and bimodal distribution are characteristic of mid-oceanic ridge heat flow patterns. Anderson and Halunen have suggested¹⁰ that metal hydroxide precipitations formed by hydrothermal exhalations within the Bauer Deep produce the metal-rich sediments there. This is borne out by our geochemical evidence based on element distributions and accumulation rates across the Nazca lithospheric plate.

The surface layers (0 to <10 cm) of 12 cores were analysed for metal content. Chemical compositions were determined by X-ray fluorescence spectrometry and atomic absorption spectrophotometry. On four of the cores, sedimentation rates were determined by the logarithmic decrease of unsupported ²³⁰Th with depth in the sediment. The core length over which the unsupported ²³⁰Th activity decreased by one-half was divided by 75,200 yr, the half life of ²³⁰Th. This method has been successful on Quaternary pelagic sediments¹¹⁻¹³. Metal accumulation rates were calculated by multiplying the bulk sedimentation rate by the metal content, assuming an *in situ* dry uncompressed bulk density for pelagic sediments of 0.75 g cm⁻³ (ref. 14).

Hydrothermal metallogenesis in the Bauer Deep of the south-eastern Pacific

SEDIMENTS from the East Pacific Rise that are unusually enriched with iron, manganese, and other metals^{1,2,3} are correlated with areas of high heat flow on oceanic ridges. With the discovery that sediments rich in iron directly overlie oceanic basaltic basement, it has been suggested that such deposits were formed by circulating hydrothermal fluids generated during mid-oceanic rift volcanism^{4,5}. Other suggested origins are: authigenic precipitation of iron and manganese from seawater⁶, submarine weathering of basaltic debris, and precipitation during diagenetic remobilisation of manganese

Table 1 Location and composition of surface sediment

Core no.	Latitude (S)	Longitude (W)	Depth (m)	CaCO ₃ (%) [*]	Al ₂ O ₃ (%) [†]	Fe ₂ O ₃ (%) [†]	MnO (%) [†]	Cu (p.p.m.) [‡]	Ni (p.p.m.) [‡]	Co (p.p.m.) [‡]	Cr (p.p.m.) [‡]
109	12°05'	110°37'	3,069	90	0.97	0.13	0.14	97	89	35	37
11	11°00'	107°30'	3,507	90	0.96	0.12	0.14	120	73	30	33
115	11°58'	103°23'	4,548	7	5.72	19.44	6.33	750	1050	210	70
121	11°59'	101°31'	3,952	77	1.93	2.15	1.01	180	200	73	48
127	12°23'	98°45'	4,062	70	3.25	3.46	1.62	310	500	70	60
132	11°33'	97°27'	3,996	75	2.99	1.93	1.00	230	370	75	39
136	12°35'	95°48'	3,457	53	7.45	6.33	2.38	470	600	100	83
138	12°36'	95°42'	4,286	5	10.05	12.28	4.16	560	950	300	95
148	11°59'	91°19'	3,945	71	4.00	2.46	0.51	260	240	150	69
151	12°01'	87°33'	4,296	17	12.97	5.97	0.99	130	390	60	120
7	10°33'	83°02'	4,514	2	13.66	6.86	0.49	160	260	29	140
162	12°01'	79°34'	5,026	3	12.88	6.27	0.10	85	38	—	135

^{*}Calculated from CaO determination by X-ray fluorescence spectrometry.

[†]Al₂O₃, Fe₂O₃ and MnO by X-ray fluorescence spectrometry. Coefficients of variation are less than 2%.

[‡]Cu, Ni, Co, and Cr by atomic absorption spectrophotometry. Coefficients of variation are less than 15%.

Figure 1 shows the location of 18 cores analysed during a larger study of their chemical and mineralogical composition (G. M. McM., unpublished). The transition metal, aluminium and calcium carbonate contents of 12 of these cores are presented in Table 1. Figure 2 shows the distribution of iron, manganese and four trace metals on a profile across the Nazca plate at approximately 12°S. The metal concentrations were normalised to aluminium in a method similar to that of Piper¹⁵, who found that the metal concentrations of East Pacific Rise sediments showed more internal consistency and exhibited stronger maxima near the rise crest when treated in that way than when plotted on a carbonate-free basis.

In the Bauer Deep, enrichment is greatest for iron, manganese, copper, and nickel (Fig. 2). Iron and manganese distributions correlate well with each other across the entire area, but those of other metals do not. Nickel and copper show greatest enrichment in the Bauer Deep and Galapagos Rise sediments, and some covariance with iron and manganese. Cobalt and chromium show no enrichment and display no covariance with the other transition metals. We interpret the strong iron and manganese covariance as evidence for coprecipitation of iron and manganese hydroxides⁹. The distributions of nickel and copper are similar to those of iron and manganese and those metals may therefore be fixed in the same manner. But hydrothermally derived iron oxides may at least partially scavenge manganese and associated metals such as nickel and cobalt from seawater by catalytic oxidation¹⁶. The distribution of chromium may be explained by its mobility in the oxidising environment of the sediments, as it forms soluble complexes in the hexavalent state¹⁷. The cobalt distribution generally follows that of chromium, although greater enrichment would be expected because cobalt should not be as mobile as chromium in these sediments.

The transition metal accumulation rates of four cores representing Quaternary sedimentation on the East Pacific Rise, the Bauer Deep, the Galapagos Rise, and on the plate near the

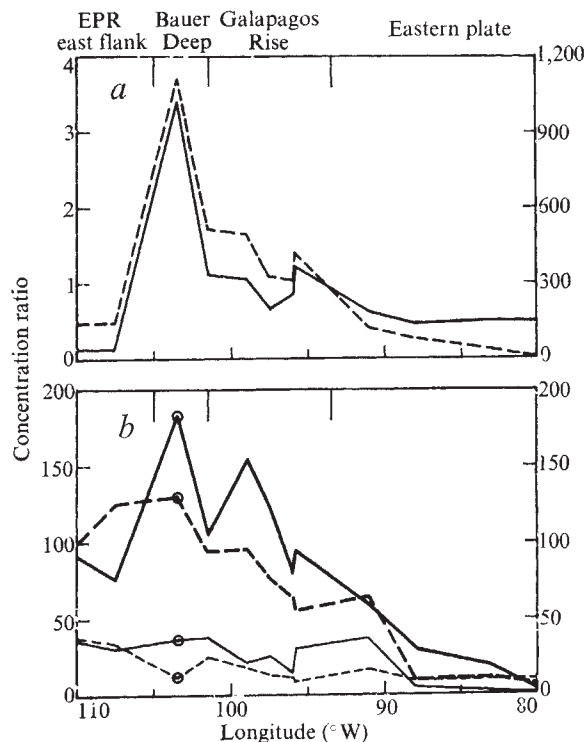


Fig. 2 Distributions of Fe_2O_3 , MnO , Cu , Ni , Co , and Cr in ratio to Al_2O_3 in surface sediments from the Nazca plate at approximately 12°S. a: — — —, $\text{MnO}/\text{Al}_2\text{O}_3 \times 10^{-3}$ (right hand scale); —, $\text{Fe}_2\text{O}_3/\text{Al}_2\text{O}_3$ (left hand scale). b: — — —, $\text{Cu}/\text{Al}_2\text{O}_3$; —, $\text{Ni}/\text{Al}_2\text{O}_3$; — — —, $\text{Cr}/\text{Al}_2\text{O}_3$; — — —, $\text{Co}/\text{Al}_2\text{O}_3$; ○, Bauer Deep Sediment. All figures in b $\times 10^{-4}$. EPR = East Pacific Rise.

Table 2 Surface sediment accumulation rates

Core no.	Region	Sedimentation rate (cm per thousand years)	CaCO_3 -free sedimentation rate (cm per thousand years)	Accumulation rates (mg cm^{-2} per thousand years)					
				Mn	Fe	Cu	Ni	Co	Cr
109	East Pacific Rise	1.08	0.11	0.88	0.73	0.08	0.07	0.03	0.03
115	Bauer Deep	0.19	0.18	6.98	19.4	0.11	0.15	0.03	0.01
132	Galapagos Rise	0.20	0.05	1.16	2.03	0.03	0.06	0.01	0.01
7	Eastern Plate	0.23	0.23	0.65	8.28	0.03	0.05	0.01	0.03

Peruvian coast are presented in Table 2. The bulk sedimentation rate across the plate is approximately 0.2 cm per thousand years except for the East Pacific Rise crest, which shows a higher rate attributable to carbonate deposition. Bender *et al.*¹⁸ determined manganese accumulation rates for pelagic sediments and nodules. They found that the manganese accumulation rates for pelagic sediments ranged from 0.2 to 3.2 mg cm^{-2} per thousand years with an average value of 1.3 mg cm^{-2} per thousand years. But an accumulation rate of 35 mg cm^{-2} per thousand years was found for a core taken farther south than ours on the East Pacific Rise crest⁶. This rapid rate of accumulation may be further evidence that manganese and other transition metals are derived from ridge-crest volcanism. Our data show that manganese is accumulating on the East Pacific and Galapagos Rises at 12°S at a rate close to the average pelagic value, but that in the Bauer Deep it is accumulating at more than five times that rate. Accumulation rates for iron, copper and nickel are similarly higher in the Bauer Deep sediments than in those from the other areas.

Our data indicate that metal-rich sediments can be derived locally in areas of great heat flow such as the Bauer Deep and need not be formed exclusively at an active spreading centre and transported elsewhere. The transition metal to aluminium maxima found by Piper¹⁵ for the East Pacific Rise crest and the

rapid manganese accumulation rate established by Bender *et al.*⁶ have been used as evidence for local volcanism as the source of these metals. Our data provide a similar argument for the Bauer Deep: iron and manganese would seem to be the direct products of volcanism. A direct relationship with volcanism is not as clear for the other transition metals.

In areas where the accumulation rates are close to those of average pelagic sediments, authigenic precipitation is probably a significant factor for metal enrichment, and submarine weathering must also make contributions in areas of slow sedimentation where authigenic minerals such as phillipsite are forming^{7,19}. Diagenetic remobilisation of manganese is unlikely, however, in oxidised pelagic sediments.

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G. M. McMURTRY
W. C. BURNETT*

Hawaii Institute of Geophysics,
University of Hawaii,
Honolulu, Hawaii 96822

*Present address: Department of Earth and Space Sciences, State University of New York at Stony Brook, Stony Brook, New York 11794.

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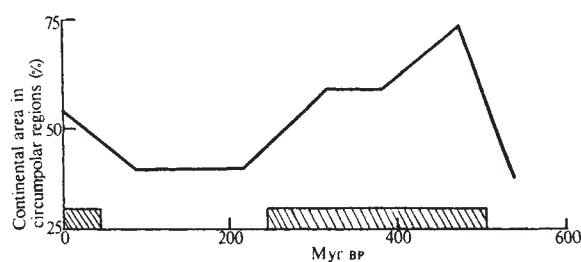


Fig. 1 Variation of continental area within 30° of poles as a function of time. The shaded parts of the time axis include all extensive glaciations^{4,5}.

Long term variations in the albedo and surface temperature of the Earth

The surface temperature of the Earth depends primarily on the solar constant, the Earth's albedo and the total mass and chemical composition of the terrestrial atmosphere. Studies of climate covering the past few million years have generally allowed for variations in albedo in calculating average values of the surface temperature. But over longer periods of time, however, less allowance has been made for albedo variations; it has, indeed, frequently been assumed that the albedo, when averaged over a long enough time, can be taken to be constant (see ref. 1). We wish to point out that, on the contrary, long term variations in the albedo can be expected to occur, and to produce significant changes in the average surface temperature.

The total albedo of the Earth depends on the relative proportions and dispositions of the continents and oceans and their related cloud covers. This varies both because the cross section presented to incident solar radiation by a given area depends on its latitudinal position, and because the variation in reflectivity with angle of incident radiation changes from land to ocean. Throughout much of the geological record, the relative amounts of continent and ocean do not seem to have undergone major change; but the relative disposition of the two has varied considerably because of continental drift. The amount of land area, as distinct from continental area, has certainly undergone fluctuations, but no systematic trend appears either in time, or relative to the disposition of the continents on the Earth's surface. We have estimated the average surface temperature of the Earth using a computer program which allows both for the greenhouse effect of the atmosphere and for variations in albedo resulting from different positions of the continental masses, but which assumes no significant change in the ratio of land-to-ocean area. (Surface temperatures have been computed as a function of latitude for land and ocean separately, and an average, weighted according to the areas involved, has then been calculated.)

If cloud cover is initially ignored, the important factor is the latitudinal distribution of the continents. We have therefore looked especially at two extreme cases: (1) where the continents are gathered together into a belt round the equator; (2) where they form a cap round one, or both, poles. We have constructed semi-empirical curves of reflectivity as a function of the angle of incident radiation, assuming in case (1) a covering of soil, and in case (2) an ice-snow covering. Although considerable information is available on the albedo of different types of surface, data on the variation of albedo with solar elevation are less well determined². Fortunately, computer runs using a range of possible albedo variations with angle indicate that only minor changes are produced in the surface temperature with any reasonable functional relationship. The difference in albedo between the two models is found to change the surface temperature by more than 12 °C (the higher temperature corresponding to the equatorial position of the continents). Since the difference between a glacial and an interglacial period probably corres-

ponds to a change in average temperature of only 5 °C (ref. 3), this albedo-dependent variation must be regarded as significant.

There are, however, two modifications that should be made. First, we have assumed extreme dispositions of the land-masses in deriving this figure for the surface temperature. More probable variations in the latitudinal position of the centre of area of the continents will still lead to differing values of the albedo, and therefore to differences in surface temperature, but the change will be smaller than that derived above. Similarly, the effect of introducing cloud cover into the calculations, for any likely cloud distribution, is to reduce the change in albedo and so the variation in surface temperature. Nevertheless, after allowing for both these factors, the amount of latitudinal change postulated in reconstructions of continental drift still seems from our estimates to be capable of producing a variation in surface temperature of a few degrees. The effect of this would be to accentuate the prevailing climatic conditions. In particular, although a land-mass near one of the poles would, in any case, be expected to have an ice-snow cover, a resultant slight lowering of the average temperature, as a result of the albedo effect, could turn this into a major glaciation. The effects of this could then extend to appreciably lower latitudes than would otherwise be the case. Whether or not this occurs will depend on other astronomical and geological factors. What we can assert is a statement of probability: that the likelihood of extensive glaciation occurring should be greater when a higher proportion of the land mass is near the poles.

We have used the data cited by McElhinny⁴ to derive values for the latitudinal distribution of the continents during each geological period. The results are best represented in terms of the percentage of the terrestrial surface within 30° of each pole which is covered by continent. The reason for choosing this form of presentation for the data is that continental displacements at lower latitudes, although leading to small changes in the albedo, cannot support extensive glaciation. It is the latter that can most readily be detected in the geological record.

Some of the data on continental drift remain uncertain. In particular, information on continental drift and major glaciations in the Precambrian is still inadequate for the type of comparison we wish to make here. Nevertheless, as can be seen from Fig. 1, if we separate out the periods during which extensive glaciations most commonly occurred⁵, these correlate reasonably well with periods when a higher percentage of the circumpolar regions was occupied by continents. Within the limits of accuracy of the data, therefore, predictions based on the albedo effect are supported by the available geological evidence.

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ANN SELLERS
A. J. MEADOWS

Astronomy Department,
University of Leicester, UK

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Recent changes in Atlantic surface temperatures

RODEWALD recently published brief notes about the temperature measurements of the sea surface made by nine North Atlantic weather ships during the past two decades^{1,2}. His remarks were prompted by the regrettable fact that several of these ocean stations are being discontinued, a decision which will make it much more difficult to monitor the temperature trends in the North Atlantic. He presented¹ average annual temperature values for overlapping 5-yr periods, showing a distinctive decrease of 0.56 °C from the 1951–55 period to the 1968–72 period (Fig. 1). Rodewald also provides² three important maps: the changes in surface temperatures from 1951–55 to 1968–72

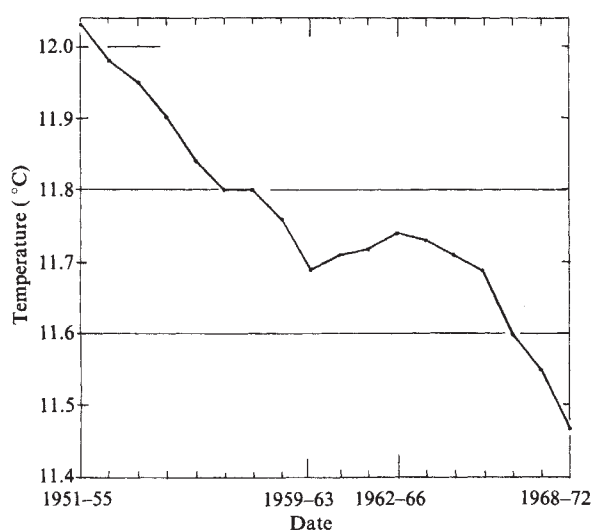


Fig. 1 Running 5-yr mean surface temperature at the nine North Atlantic fixed station weather ships, according to Rodewald¹.

for the annual mean, and for the months of February and August. Figure 2 shows his analysis of the February changes. There is a remarkable consistency in the values; for example, at Station D (44°N, 41°W) the surface temperature declined by 1.61 °C in the annual average, by 2.12 °C in February and by 2.10 °C in August. These are surprisingly large changes over such a short time span. Since they are based on a well defined homogeneous record of careful observations there can be no doubt that they are real. The core of decreasing temperature lies along the north edge of the Gulf Stream extension, suggesting a south eastward shift of that current and replacement with Labrador Current waters, especially in the vicinity of the Grand Banks. The largest changes are encountered across the Atlantic between 40° and 50°N.

Here we attempt to put Rodewald's maps into perspective by comparing them with maps of surface temperature change for other time intervals, so that one can assess the significance of the changes he depicts. One of these may be obtained by subtracting the temperature pattern obtained by the CLIMAP group³ for the most recent ice age (approximately 18,000 yr ago) from the recent pattern (Fig. 3). The CLIMAP pattern was constructed by objective methods from the assemblages of fossil planktonic foraminifera in approximately 90 deep-sea cores.

Values north of 50°N could not be determined with great accuracy as, according to the ice-age map, temperatures there are given as 'below +2 °C'. The differences cannot, however, be much larger than approximately 12 °C near 45°N and less farther north, since the current temperatures at these latitudes are not higher than +12 to +13 °C. The CLIMAP charts from which Fig. 3 was derived clearly show that the long tongue of maximum anomaly along 45°N results from a southward shift of the maximum temperature gradient at the north edge of the Gulf Stream extension.

In Fig. 2, the values of the 22-yr change in February temperatures given by Rodewald are entered at the various weather ship station locations. At Station D, for example, (indicated also on Fig. 3) the 22-yr recent change of February surface temperature was –2.12 °C. This station is located in the area of maximum change between the most recent ice age and today; the estimated change was about 11°C. So it seems that we have experienced, in the past 29 yr or so, a change in Atlantic ocean temperatures which amount, in the Gulf Stream vicinity, to about one-sixth of the difference between total glaciation and our present climate.

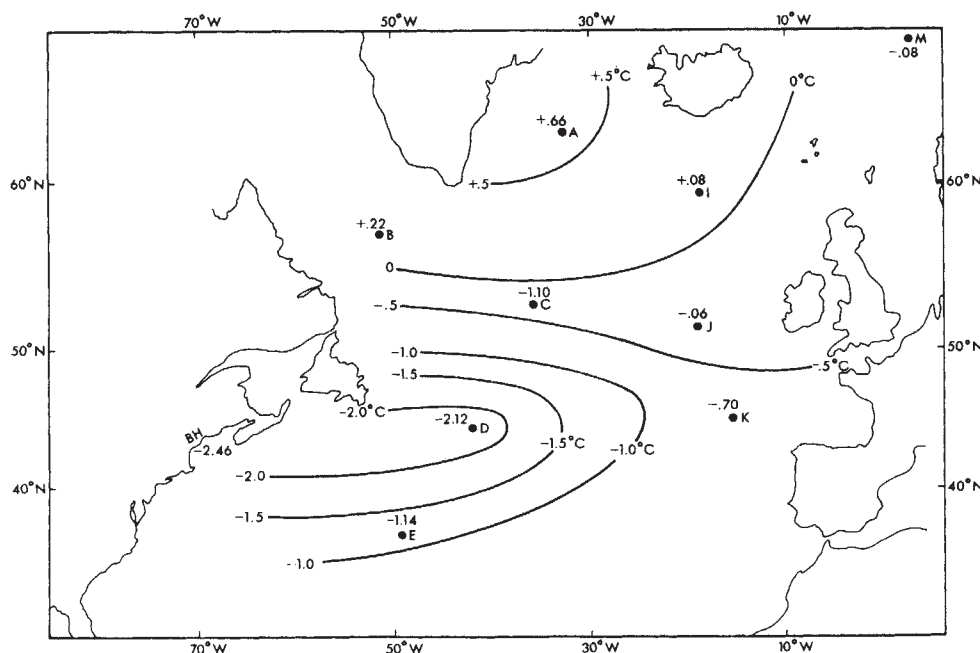


Fig. 2 Change of February North Atlantic surface temperature from the 1951–55 pentade to the 1968–72 pentade, according to Rodewald². (Data for this figure would support an analysis even more consistent with that of Fig. 3 than Rodewald's analysis.)

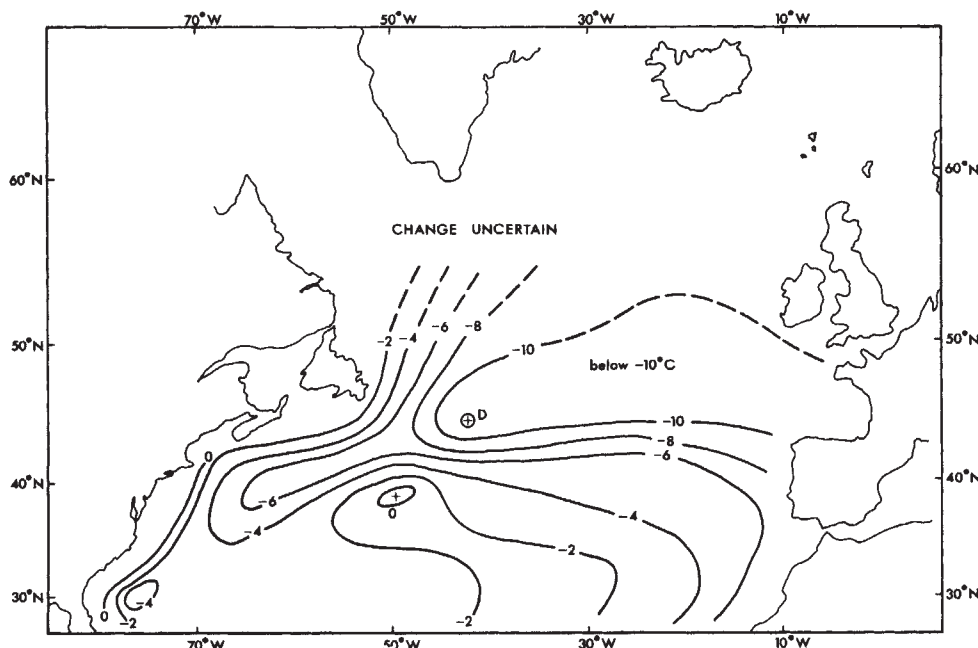
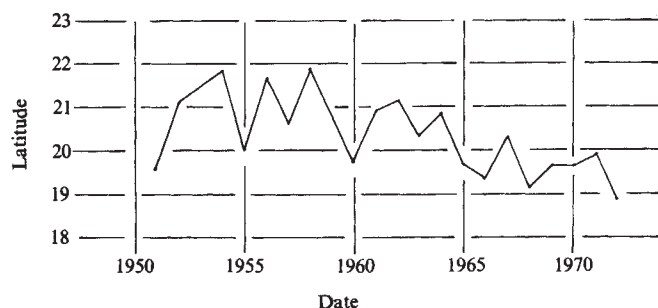


Fig. 3 Difference between ice age surface temperatures, (approximately 18,000 yr ago) and present winter surface temperatures, derived from the CLIMAP reconstruction³.

Another comparison may be made with the map of average North Atlantic surface water temperature change between the early 19th century and the 1887–1899, 1921–38 period given by Lamb⁴. Rodewald's figures approximately mirror those given by Lamb, indicating that the change from 'Little Ice Age' conditions to the early part of the present century was just about wiped out between 1951 and 1972. The 'Little Ice Age' thus seems to have been a return of about one-sixth of the way to a full ice age. Rodewald's map in turn indicates that, at least as far as surface temperatures in the North Atlantic are concerned, the pattern had returned roughly to 'Little Ice Age' conditions from its early 20th century excursion by 1972.

Two other pieces of information tend to confirm the relative magnitude of the surface temperature change given by Rodewald. First, in West Africa during the same 1951–72 period, the northern edge of the monsoon drifted irregularly southward, as indicated in Fig. 4. Assuming that if this displacement persisted at the 1968–1972 latitude the biotic zonation would adjust similarly, a comparison may be made with the late glacial zonation as given by Cloudsley-Thompson⁵. Once again the 1951–72 shift seems to have been about one-sixth of the late glacial to present displacement.

Fig. 4 Northward penetration of the monsoon rains in West Africa in the longitudes of Nigeria. The higher the latitude to which the rains penetrate, the greater the seasonal rainfall. The latitude of the northern edge of the monsoon rains was obtained by least squares fitting of a trend line to the rainfall amounts at various stations in the longitudes of Nigeria. The intercept of this line with zero rainfall was taken as the northern edge of the monsoon. Few stations north of this calculated position reported more than a few millimetres of rainfall. Plotted points are averages of the July and August values.



The second observation is the change of worldwide annual average snow and ice cover reported by Kukla and Kukla⁶. In the northern winter of 1971–72 there was a rapid increase of snow and ice cover which persisted into the following years. This increase was also about one-sixth of the difference between present-day conditions and those at the end of the most recent glaciation.

We do not have an adequate basis for estimating whether or not the changes observed in the 1951–72 period will continue to grow, but it is essential to monitor further development with great care. Indeed, we do not know whether the pattern of 1974 represents an end to the trend of the previous two decades or simply a temporary reversal like those which have characterised individual years of those decades on occasion. But we should not assume that the change indicated by Rodewald's data is just a short term passing anomaly easily reversed in a year or so.

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E. W. WAHL
R. A. BRYSON

Center for Climatic Research,
Institute for Environmental Studies,
The University of Wisconsin,
Madison, Wisconsin 53706

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Buckle propagation in submarine pipelines

DURING its construction a submarine pipeline is at the same time bent and loaded by the external pressure of the sea. If it is bent too far, it buckles. If the local external pressure is small, the buckle is limited to a short kink on the compressed side of the pipe. But if the external pressure is large enough, the local bending buckle can transform itself into a buckle of a different form, which once initiated can propagate along

the pipe, driven by external pressure alone, even into regions free of bending. Figure 1 shows a pipe along which a buckle has propagated in this way. The mode can be observed by grasping a tube of toothpaste firmly between thumb and forefinger, and then moving the fingers along the tube. In a short transition region, between the fingers, the pipe bends from a circular into a dumb-bell cross section.

This problem is important because of the possibility that a mishap during pipelaying might initiate a buckle which would run along the completed section of a pipeline, perhaps for many kilometres, at a cost of the order of \$1.5 million a kilometre. Little has been published about this problem¹. We describe the results of a preliminary analysis and of some tests on model pipes, and concentrate on determination of the minimum pressure needed just to sustain propagation. Buckle



Fig. 1 Pipeline collapsed by a propagating buckle. The test was carried out on steel pipe of outside diameter 813 mm and wall thickness 19.1 mm.

propagation of this kind is distinct from the classical phenomenon of elastic buckling of cylinders under external pressure²; in a typical pipeline the propagation pressure is about a third of the elastic critical pressure.

A pipeline is essentially a steel tube with radius:thickness ratio of the order of 20, cold formed from a medium strength ductile steel and surrounded by a concrete weight coating (which contributes little to its strength). It can be treated as a thin shell. We begin by considering the kinds of deformation such a shell can undergo. It can either deform inextensionally, in a mode which bends the middle surface but does not stretch it, or extensionally, in a mode which both stretches and bends the middle surface. In general, the amount of energy required to deform the shell in an inextensional mode is much less than that to produce a deformation of similar magnitude in an extensional mode³; the shell 'prefers' to deform inextensionally if it can. Rayleigh showed, however, that if a cylindrical shell is constrained so that one circumference undergoes no deformation, then no purely inextensional deformation is possible³. Although there is thus no way in which the buckle can propagate without any stretching of the middle surface, it will develop a buckle configuration in such a way that the pipe is distorted by bending as much as possible and stretching as little as necessary.

An estimate of the propagation pressure can be made by assuming a buckle to move along the pipe in an unvarying mode (so that an observer moving with it would always see the same buckle configuration), and equating the work done by the external pressure on the consequent volume change to the work required to deform the pipe. Since the deformation is so large, plastic deformation dominates over the effects of

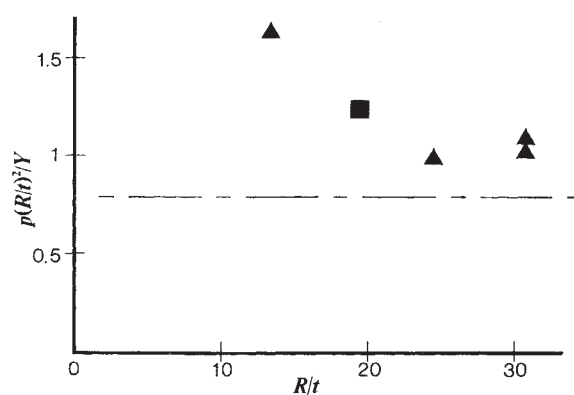


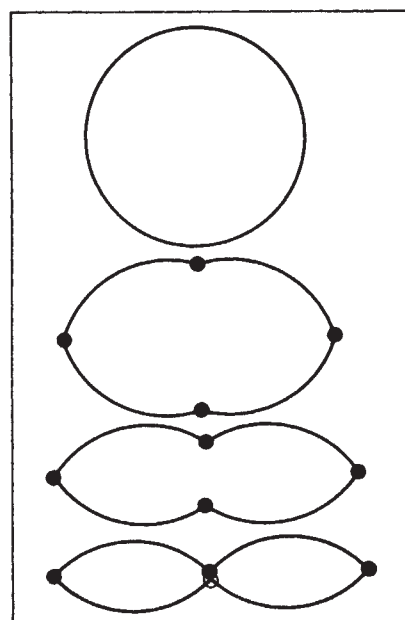
Fig. 2 Dimensionless propagation pressure $p(R/t)^2/Y$ as a function of R/t . Points indicate experiments on stainless steel tubes of diameter 50.8 mm (■) and 12.7 mm (▲). — — —, Predicted by simple theory.

elasticity, so that the pipe deforms almost as it would if it were made from a rigid-plastic material. Then if the mode of deformation is purely inextensional, the propagation pressure p will be proportional to Yt^2/R^2 , where Y is the yield stress, t the wall thickness of the pipe and R its radius. Different pipes made from different materials can then be compared by evaluating the non-dimensional propagation pressure $p(R/t)^2/Y$, which will be a constant if extensional effects are not important, and a function of R/t if they are.

Figure 2 shows the results of tests in which a buckle was driven along steel tubes. It shows that different pipes may be compared in this way, and suggests that the presence of extensional deformation exerts little influence for R/t greater than 20.

A theoretical estimate of the propagation pressure can be made from the work calculation described earlier. Suppose a cross section of the pipe to deform in the way shown in Fig. 3. All the deformation is in bending, concentrated into four 'plastic hinges'⁴, separated by rigid regions. Two opposite hinges move together until they meet, and the other two move

Fig. 3 Mode of collapse assumed in analysis.



apart. If the material is perfectly plastic, the work calculation gives

$$p(R/t)^2/Y = \pi/4$$

Comparison with Fig. 2 shows that this somewhat underestimates the observed pressure. Work calculations of this kind usually overestimate plastic collapse loads, and indeed can be proved to do so in the case of small deformations. Here the deformation is not small, and the buckle must also obey an internal equilibrium condition. If the transition from the circular to the dumb-bell cross section were very long, extensional deformation would be negligible and the total work dissipated would approach that corresponding to the above equation. But more detailed examination of the forces within the pipe wall shows that a buckle with such a long transition cannot propagate, because its equilibrium would require axial forces too large for the pipe wall to carry. Instead the transition shortens until the equilibrium conditions can be met: this involves a steeper transition, more stretching deformation, and a larger propagation pressure.

Other modes than the one shown in Fig. 3 can be used in the analysis, but this one gives the lowest propagation pressure. Its final cross section agrees quite well with the one observed in experiments.

One way of avoiding the danger of pipeline collapse by buckle propagation is to design the pipe so that its propagation pressure is greater than the greatest external pressure the sea can exert. It may instead be more economical to use a thinner wall but to guard against the possible destruction of long sections of line by placing at regular intervals 'buckle arresters' which will stop buckles which have begun to propagate. The design of buckle arresters will be discussed elsewhere.

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ANDREW C. PALMER
J. H. MARTIN

University Engineering Department,
Trumpington Street, Cambridge, UK

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Xenopus from the Palaeocene of Brazil and its zoogeographic importance

THE African frog *Xenopus* (a member of the family Pipidae), formerly found in Miocene^{1,2} and Recent deposits in Africa, is described here from the Palaeocene (~ 60 Myr) of Brazil. Extant pipids are aquatic freshwater frogs, occurring in sub-Saharan Africa (*Xenopus*, *Hymenochirus*, *Pseudhymenochirus*) and northern South America (*Pipa*, *Protopipa*, *Hemipipa*). They display a mosaic of primitive and specialised characteristics and are generally placed as an ancient offshoot of early anuran stock. Both fossil and recent occurrences of the Pipidae are consistent with an ancient distribution exclusively across the southern continent^{3,5}.

The Palaeocene material from Brazil consists of well preserved, uncrushed braincase and postcranial elements, including those diagnostic of *Xenopus*—relatively narrow, flat, and unsculptured skull roof bounded by parasagittal crests; well defined parietal foramen; paired or unpaired vomers, occasionally toothed; three or occasionally four acoustic foramina in the medial braincase wall; dagger shaped parasphenoid, lateral wings absent; only the inferior perilymphatic foramen present; cleft scapula; fused scapula and clavicle; iliac shaft lacking crests.

The morphological characteristics (expanded ear capsules, azygous frontoparietal, presence of parietal foramen, bilobed trochlear articulation of humeral head, far lateral position of the inferior perilymphatic foramina, short scapula, opisthocelous (probably epichordal) vertebrae, fused sacrum and urostyle, and sacrum with widely expanded diapophyses) indicate that this frog is a member of the family Pipidae. The general head shape and the listed diagnostic features confirm our reference to the distinctive genus *Xenopus*, limited today to sub-Saharan Africa. Extant South American pipids and the African *Hymenochirus* are characterised by broad flattened skulls with a generally extensive supraorbital shelf; anteriorly wide parasphenoid; the presence of only the superior perilymphatic foramen and only a single acoustic foramen; a separate clavicle and scapula; and widely expanded iliac shafts. The distinctive morphology of pipids in general and of *Xenopus* in particular leaves no doubt about the identification.

Classification and description of Brazilian specimens

Class Amphibia
Subclass Lissamphibia
Order Anura
Superfamily Pipoidea
Family Pipidae

Xenopus romeri, n. sp.

Holotype: DGM (Divisão de Geologia e Mineralogia) 568, braincase and sphenethmoid region of skull.

Paratypes: DGM 569, partial skull; 570, braincase; 579, ethmoid region; 575a-e, vertebrae; 576, fused first and second vertebrae; 573, sacrum with fused urostyle; 571, scapula-clavicle; 572, humeri; 577-578, ilia.

Locality: fissure deposits in limestone in the quarry of the Companhia Nacional de Cimento Portland (Mauá), São José de Itaboraí, Estado Rio de Janeiro, Brazil⁴. Specimens collected in 1968.

Age: late Palaeocene.

Etymology: the species name honours the late Professor Alfred Sherwood Romer for his work on relationships of African and South American fossil faunas.

Diagnosis: species of *Xenopus* with robust, widened, well ossified skull with extensive fusion among braincase elements; cultriform process of parasphenoid fused or unfused; nasals co-ossified, may or may not be fused to frontals; parietal foramen opening far anteriorly on frontal; retractor bulbi muscle scars not excavating the parasphenoid ventromedially; a well defined foramen for internal carotid artery present anterior to palatine foramen; a well ossified prepalatine connection separating palatine foramen from pro-otic foramen; three acoustic foramina (four in one specimen); condyloid fossa relatively well developed, with inferior perilymphatic foramen opening posteriorly into the latter and separated dorsally from jugular foramen by a distinct roof or crest; parasagittal crests relatively far apart and meeting posteriorly almost at posterior border of frontoparietal; dorsal protuberance of ilium relatively small; first and second vertebrae fused; zygapophyseal surfaces simple.

Description: description here includes only those features necessary to confirm identification, or those that are of special anatomical interest. In DGM 568 (type) nasals and vomers are lost, not having been co-ossified (Fig. 1); in DGM 569 vomers and nasals are fused to the skull (Fig. 2), with faint or no indication of sutures.

The trigeminal foramen is separated ventrally from palatine foramen by well ossified prepalatine connection; anterior to the palatine foramen a well ossified pillar of bone sets off an internal carotid foramen ventrally. On the medial braincase wall (visible laterally on 569 because of breakage) three prominent acoustic foramina occur (four on 569, see Fig. 2C); dorsal to the acoustic foramina a prominent endolymphatic foramen occurs. In occipital view inferior perilymphatic foramina open posteriorly, separated dorsally from jugular foramina by distinct crests.

Vertebrae opisthocelous; centra flattened, probably epichordal. Identified specimens include vertebrae 1-2 (fused), 3-6, 8-9; urostyle fused to sacrum; sacral diapophyses strongly expanded. Scapula and clavicle fused; scapula short, a prominent cleft present. Humerus with well ossified proximal end showing trochlear articulation characteristic of pipids, distal ends broken. Iliac with long, subcylindrical shafts, low rounded dorsal protuberance, prominently crested acetabulum, and strong interiliac symphysis region.

Interrelationships among extant species of *Xenopus* have not been studied extensively; it is a relatively well defined, compact genus but there are few known osteological features that can be used to separate each species. Lengthy comparisons cannot be made here, but the Brazilian fossil *Xenopus* shows no special resemblance to the extant species *X. laevis*, *X. clivii*, *X. mulleri*, *X. fraseri*, and *X. gilli* (material of *X. kisigiensis*⁵ was not available for this study).

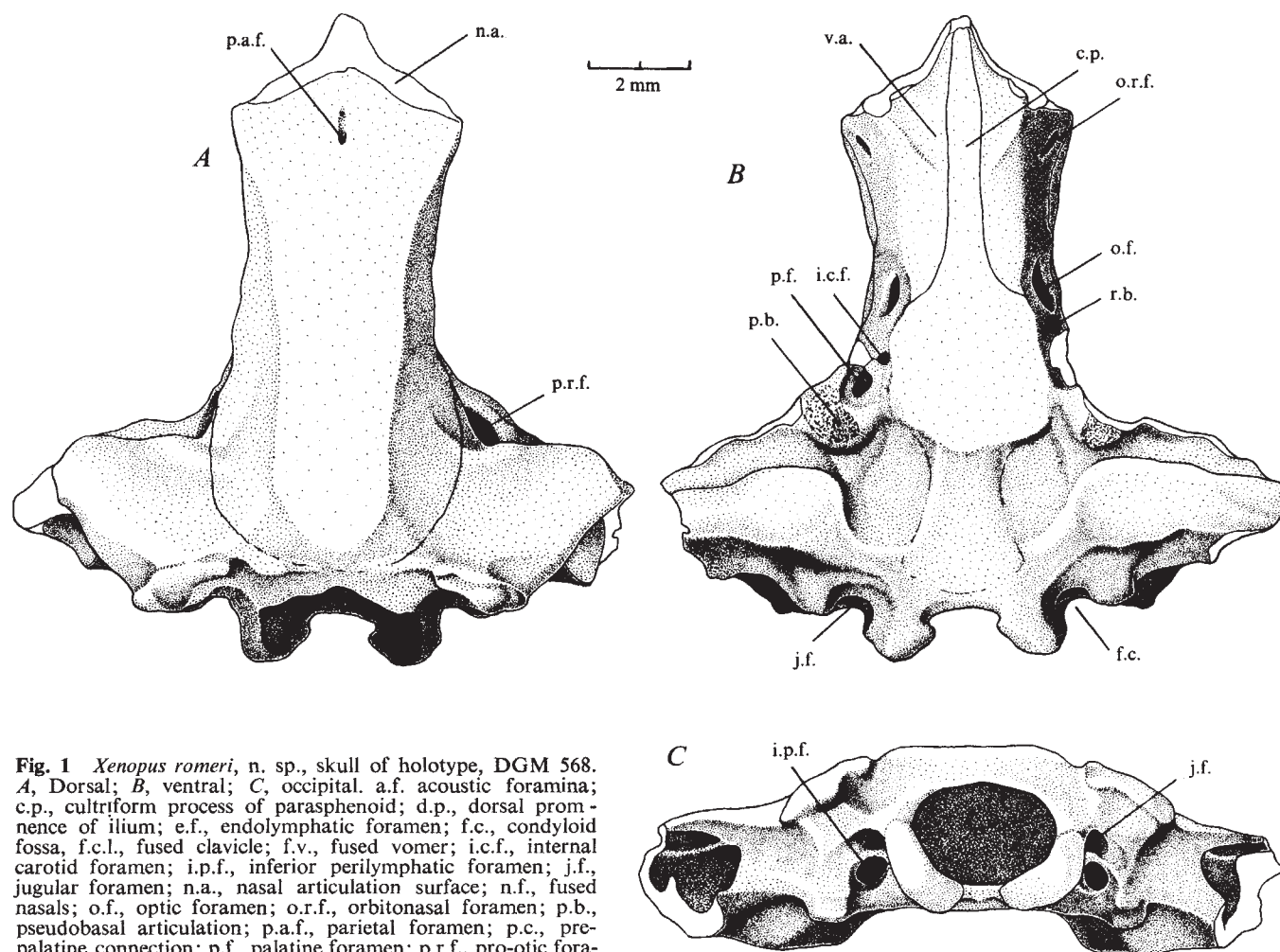


Fig. 1 *Xenopus romeri*, n. sp., skull of holotype, DGM 568. A, Dorsal; B, ventral; C, occipital. a.f., acoustic foramina; c.p., cultriform process of parasphenoid; d.p., dorsal prominence of ilium; e.f., endolymphatic foramen; f.c., condyloid fossa, f.c.l., fused clavicle; f.v., fused vomer; i.c.f., internal carotid foramen; i.p.f., inferior perilymphatic foramen; j.f., jugular foramen; n.a., nasal articulation surface; n.f., fused nasals; o.f., optic foramen; o.r.f., orbitonasal foramen; p.b., pseudobasal articulation; p.a.f., parietal foramen; p.c., prepalatine connection; p.f., palatine foramen; p.r.f., pro-otic foramen; r.b., retractor bulbi muscle scars; t.f., trochlear foramen; v.a., vomerine articulation surface.

So far as fossil *Xenopus* are concerned, *X. romeri* and the Miocene *X. stromeri* from South-west Africa¹ both have the parietal foramen in a far anterior position and relatively small occipital condyles, so far as may be judged from the rather poor figures of the latter (unfortunately the materials of *X. stromeri*, deposited in the Humboldt Museum in Berlin, have been lost). More significantly, *X. romeri* resembles an unnamed Miocene *X. tropicalis*-like form from Morocco² in having a relatively short centrum and closely-spaced glenoid cavities of the fused first and second vertebrae. These conditions are of special interest as there are a number of resemblances between *X. romeri* and the living *X. tropicalis* from western Africa; a greater number than with other living or fossil species. In general, the length-width proportions of the braincase region (especially the greater width of the braincase at the level of the orbitonasal foramen), the degree of fusion and the extent of ossification of the braincase elements (including the dermal elements), the posterior extent of skull table, the position of retractor bulbi scars, and the fusion of the first two vertebrae, indicate a similarity or close resemblance between the two species.

Although *Xenopus romeri* has a number of unique features that set it aside from other species of *Xenopus*, the number of resemblances to *Xenopus tropicalis* suggests that there is an actual relationship between *X. tropicalis* and *X. romeri*. Vergnaud-Grazzini² has suggested that the similarities to *X. tropicalis* shown by her Miocene Moroccan specimens are convergent rather than indicative of relationship. Since *X. romeri* seems to bridge the gap between the Moroccan fossils and the extant *X. tropicalis* in the configuration of the fused first and second vertebrae, it is possible that these three groups

may be more closely related *inter se* than to other species of *Xenopus*.

The presence of the now extinct *Xenopus romeri* in South America is of considerable zoogeographic interest. It is compatible with the present plate tectonic theories: South America and Africa did not separate completely until sometime in the Cretaceous^{6,7}. The presence of *X. romeri* in South America and its close resemblance to the extant western African species *X. tropicalis* is strong biological evidence for a past connection between the two continents. Similar (although weaker) evidence from the same locality in Brazil is offered by *Apodops pricei*, the first known fossil caecilian amphibian, the relationships of which seem to be with a living genus from western Africa⁸.

Knowledge of the southern (Gondwanan) radiation of pipids thus forms a more coherent picture. *Xenopus* is known now from South America as well as from Africa, and both of these continents also include forms that show intermediacy between *Xenopus* and the extant South American pipids: the Cretaceous *Saltenia*⁹ in South America and the living *Hymenochirus* in Africa¹⁰. These resemblances cannot be explained by the migration of several now extinct and unrepresented phyletic lines through northern continents; they are clearly remnants of distribution on a single continental mass.

Both of the Miocene occurrences of *Xenopus* in Africa indicate that groups resembling the extant *X. mulleri* (*X. stromeri*) and *X. tropicalis* (the Moroccan fossils) were well established by that time. The resemblance of the latter two species to the Palaeocene *X. romeri* indicates that radiation within *Xenopus* must have preceded separation of the African and South American continental plates. There exists some minor disagree-

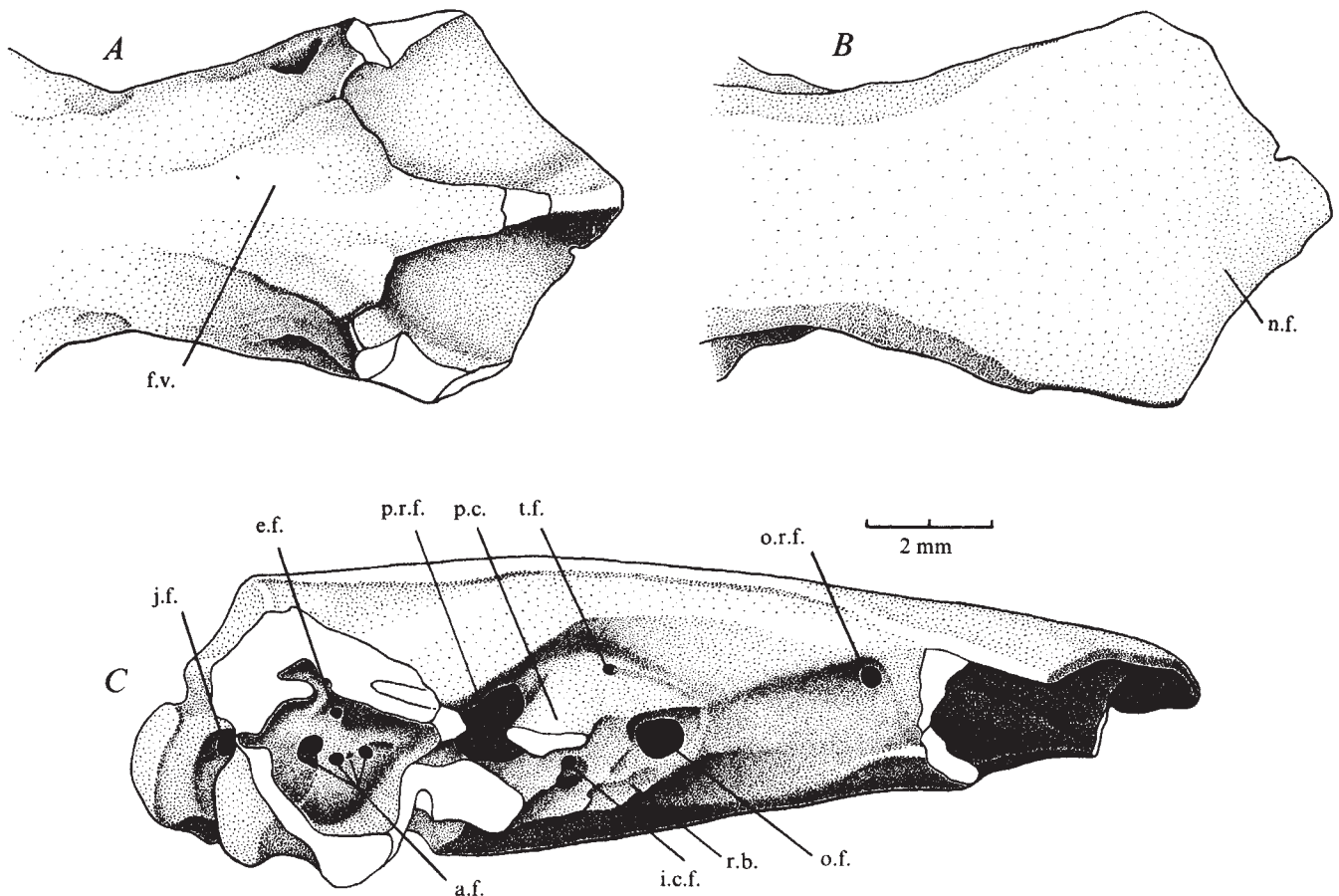


Fig. 2 *Xenopus romeri*, n. sp., DGM 569. A, ventral view of snout; B, dorsal view of snout; C, right lateral view of skull. Abbreviations as in Fig. 1

ment with regard to the timing of the separation, but available evidence indicates that a date of 120 Myr (early Cretaceous: Neocomian) for the onset of plate separation is consistent with a date of 90 Myr (late Cretaceous: Turonian) for the actual separation of the continents by a water gap. By about 70 Myr ago (late Cretaceous: Maastrichtian) there seems to have been a gap large enough to have prevented east-west migration of marine reef colonies⁴. *Xenopus* tadpoles can tolerate some salinity¹¹, but in view of the aquatic habits of the genus such tolerance would probably not be sufficient to permit migration over an oceanic barrier, even on a large raft of floating vegetation. It thus seems possible that species of *Xenopus* not much more primitive than *X. romeri* (and therefore closely related to extant species) had developed by at least 70 Myr ago, and possibly as far back as 95 Myr ago¹².

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RICHARD ESTES

Department of Zoology,
San Diego State University,
San Diego, California 92182

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Physical basis and ecological significance of iridescence in blue plants

MANY terrestrial plants of lowland tropical rainforests exhibit a conspicuous blue-green iridescence on their leaves—Richards¹ has observed these plants in Africa, South America, and South-east Asia. We have seen many species of blue plants on the rainforest floor in Malaya, from diverse groups including the ferns, *Selaginella*, and flowering plants. In Malaya the most spectacular and most common iridescent blue plant is *Selaginella willdenovii* (Desv.) Spring, also frequently cultivated in greenhouses. All comments on the iridescent colour refer back to the observation of Stahl² who reported the presence of reflection granules in the epidermal cells of *S. willdenovii*.

Although there has been ample research on iridescent colour in animals, virtually nothing has been done with plants. The iridescent green colour of the cave moss, *Schistostegia*, has been explained by the presence of peculiar epidermal cells which act as refractive lenses, focusing light on specially

oriented chloroplasts³. Here, we propose an explanation for the iridescent blue colour in plants (*S. willdenovii* in particular), provide evidence supporting our hypothesis, and speculate on the physiological and ecological significance of this iridescent blue colour in shade-tolerant herbaceous tropical plants.

Stahl's early conclusions about plant iridescence can be refuted by simple observation. First, the existence of granules reflecting blue light cannot be verified by microscopic observation. Secondly, the iridescent colour disappears when the leaves are immersed in water. Therefore, the colour must be an optical effect of the leaf surface and not of the internal structure. The two optical phenomena that can provide a physical basis for this effect are diffraction and thin-film interference, both of which have been invoked to explain the iridescent colouring of many animal cuticles^{4,5}. Diffraction effects can be ruled out in the present case because there is no dispersion; reflected blue colour is constant for white incident light over a wide range of angle of incidence. Furthermore, microscopic examination has revealed no surface features that could function as a grating. Therefore, we suggest that the iridescent blue colour in *S. willdenovii* is caused by a thin-film quarter wavelength interference filter on the upper cell wall of the epidermis. This would account for previous observations; and direct experimental support has been obtained by analysing the reflectance of light from the leaves. The spectral analysis was carried out using a Beckman Acta V spectrophotometer with total fluorescence attachment. An opaque blank was used to project a monochromatic beam of light on to the leaf (at an incident angle of 60°) and to measure its intensity reflected (at the same incident angle) into the photocell. A slit programme and a wavelength interval of 300–700 nm were used for all measurements.

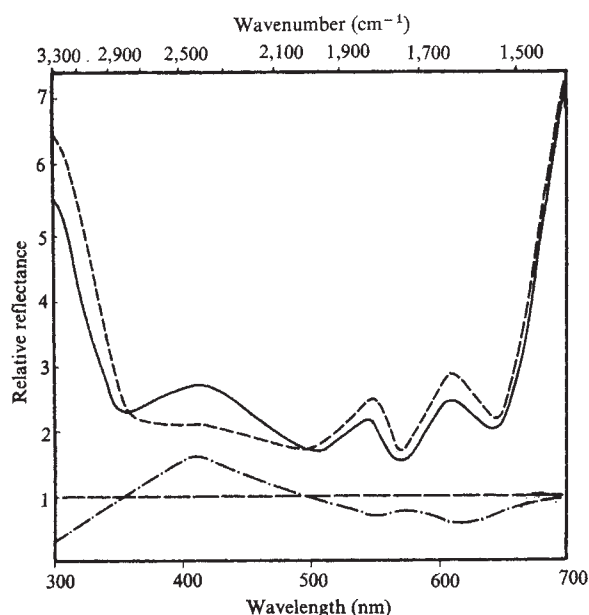


Fig. 1 Reflectance spectra of *S. willdenovii* leaves. Reflectance of the iridescent leaves is indicated by the solid line, of the non-iridescent leaves by the dashed line, and the difference between the two by the dashed and dotted line. The horizontal dashed line indicates wavelengths of enhanced reflection or transmission. Reflectance units are relative, and based on response of the spectrophotometer on a scale of percentage transmission.

When iridescent leaves age or are exposed to sunlight for some time they lose their iridescence and develop an ordinary green appearance. Chlorophyll content was found to be the same in both types of leaves (12 mg per g dry weight, by a colorimetric determination). We measured the reflectance of both iridescent and non-iridescent leaves, and plotted a difference spectrum (Fig. 1). The difference spectrum shows maximum enhanced reflectance at 405 nm, a null point at

500 nm, and decreased reflectance at longer wavelengths; the effect was obscured above 660 nm by the strong reflective characteristics of all leaves^{6,7}. This curve corresponds closely with the operation of a quarter wavelength interference filter. Precise analysis of the curve is only possible if the refractive index (n) of the filter is known. We were not able to determine this, but estimate the filter thickness at 135–150 nm (assuming $n = 1.4$ – 1.5). Electron microscopic analysis would verify the existence of such a structural layer on the wall surface. Solubility experiments suggested that cuticular waxes were not acting as the filter; the structural basis may well lie in cellulose orientation at the wall surface.

General field observations suggest the functional significance of iridescence in certain plants. Iridescent blue plants from distantly related groups grow exclusively in extremely shady tropical forest environments. Furthermore, we have observed that leaves of *S. willdenovii* lose their iridescence when they grow in sunlight, even when shade-green leaves of the same plant have the iridescent colour. The light climate on the floor of the rainforest is extremely limiting for plants; the light intensity is low and deficient in wavelengths important for photosynthesis^{10–12}. The ecological importance of an interference filter in these circumstances is that the increased reflection of photosynthetically less active light (400–500 nm) is accompanied by increased penetration in the most photosynthetically active range (600–680 nm); this would have definite adaptive value. Finally, preliminary observations on the leaf anatomy of *S. willdenovii* indicate that the epidermal cells of these plants—egg-shaped with convex outer surface and chloroplasts in a peculiar position distal to the surface—may also function as lenses in a manner similar to that found in the moss *Schistostegia*⁸. Thus the analogy of a camera with coated lens may aid our understanding of the function of the leaf surfaces in these iridescent plants.

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D. W. LEE
J. B. LOWRY

Faculty of Science, University of Malaya,
Kuala Lumpur, Malaysia

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Sex recognition pheromone in tsetse fly *Glossina morsitans*

ATTEMPTS to identify a sex attractant substance in tsetse flies have failed because there seems to be no olfactory agent for sex recognition^{1,2}. Males are apparently sexually activated only by movement of the female, but the adverse effects of naturally occurring low population densities³ on mating frequency is overcome as both sexes encounter one another at relatively high density around host animals¹. Experience in the laboratory confirms that sexual arousal in male tsetse flies, *Glossina morsitans morsitans* (Westwood), is initiated by movement of other individuals. Thus, mature adult males will initiate sexual behaviour in the absence of females and the level of activity is increased if the flies are disturbed. This behaviour ceases, however, as soon as physical contact is made with the target

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Protection from habituation by lateral inhibition

HABITUATION, or response decrement, functions to reduce an animal's responsiveness to maintained or frequently repeated stimuli, while ensuring high responsiveness to novel stimuli. Under some circumstances, however, habituation is inappropriate, in response to sensory afference caused by an animal's own activities, for example. Mechanisms for protection from habituation under such circumstances are likely to be common. Here we show, in locust movement detector (MD) neurones, that whereas habituation is rapid in response to repeated small field movements it is absent in response to maintained whole field movements. Prolonged whole field movements do not therefore reduce the responsiveness of the MD neurones to subsequent small field movements. Protection from habituation is achieved by lateral inhibition which acts prior to the site of response decrement to suppress the response to whole field stimuli.

Part of the neuronal circuitry which mediates visually

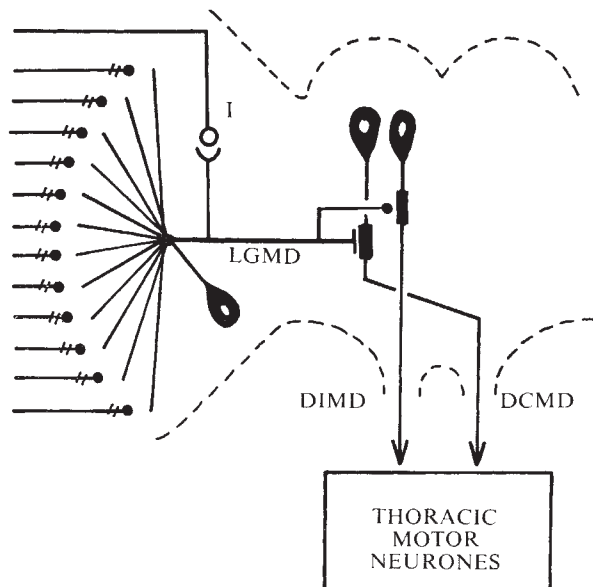


Fig. 1 Highly diagrammatic representation of the anatomical relationships of the MD system. The LGMD has a fan-shaped arborisation in the lobula of the optic lobe. Its spikes transmit along its axon into the brain, where the neurone forms an electrical synapse with the DCMD neurone and an excitatory chemical synapse with the DIMD neurone. Both these neurones run to the thoracic ganglia and make synaptic contact with motor neurones involved in jumping. The main fan of the LGMD is the site of afferent synapses from the medulla, which supply a processed retinal projection to the LGMD. The excitatory chemical synapses are markedly labile and habituate readily (indicated by double cross-hatching). The LGMD also receives, nearer the site of initiation of spikes, an inhibitory input (I) which derives its input from spatial summation over the whole of the visual field of the eye, and which is responsible for post-convergence inhibition in the system.

induced escape jumping in the locust has been elucidated by intracellular recordings from identified interneurons and motoneurons¹⁻⁴. Three bilaterally paired MD neurones respond vigorously to abrupt movement of small and contrasting objects anywhere in the visual field. They are, however, almost insensitive to whole field movements⁵⁻⁷. The lobular giant movement detector (LGMD) of the optic lobe provides the main input to two cerebral units, the descending contralateral movement detector (DCMD) and the descending ipsilateral movement detector (DIMD) which relay spikes from the brain to the metathoracic motoneurons responsible for jumping. The anatomy and major connections of the MD system are summarised diagrammatically in Fig. 1.

The LGMD is most responsive to new movement and derives this ability from rapid decrement of the response (habituation) produced when the same part of the retina is repetitively stimulated^{8,9}. As decrement is limited to the stimulated area, and as the fan-like arborisation of the LGMD is the site of convergence of the afferent pathways, habituation must occur presynaptically to it. This is confirmed by intracellular recordings from the LGMD, which suggest that the habituation occurs presynaptically at the afferent synapses on the LGMD fan.

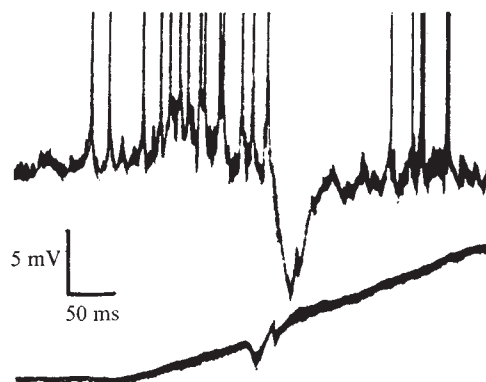


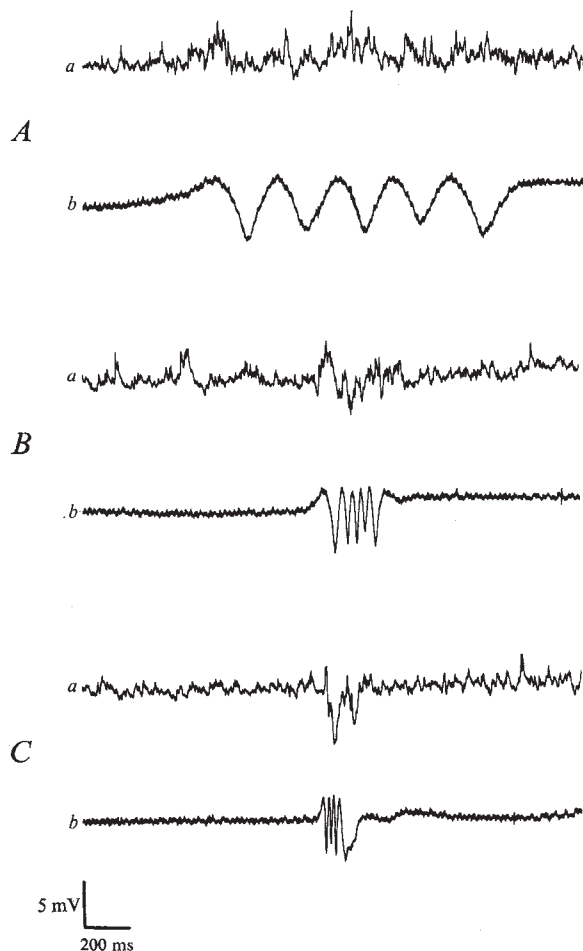
Fig. 2 Postconvergence inhibition in the LGMD of the response to a small moving target, produced by rapid displacement of the whole visual field. The upper trace is an intracellular record (scale 100 ms and 5 mV) from the fan of the LGMD. The slow ramp in the lower trace is the voltage analogue of the movement across the visual field of a small (about 10°) black target, which produces a large barrage of EPSPs and spikes in the LGMD. The background consists of a stationary array of vertical black and white stripes covering the whole visual field. About halfway through the response, the background is suddenly moved laterally, at a speed corresponding to 80 light-dark or dark-light transitions at the individual retinal receptor per second, for a period of 50 ms. This is indicated by inflections on the ramp. The movement of the background produces a strong hyperpolarisation which suppresses the response to small-field movement.

Although there is little or no response to whole field movement the illumination of most or all of the ommatidia of the retina changes under such circumstances, depending on the complexity of the visual environment. Palka⁸ showed that large or whole field stimuli evoked both excitation and, preponderantly, inhibition in the MD system. He found that the inhibitory effect of such a stimulus to one retinal area affected the response of the other areas equally, and not in proportion to their remoteness, as would be expected of a lateral inhibitory network. We have corroborated Palka's results, and by intracellular recording shown the basis of the inhibition to be large summing inhibitory postsynaptic potentials (IPSPs) in the LGMD, which occur in response to rapid changes of illumination affecting large areas of the retina (Fig. 2). That this type of inhibition acts proximally to the site of convergence is consistent with the observation that its effect is equal over the whole visual field.

If this postconvergence inhibition was the only mechanism suppressing the response to whole field stimuli, all labile

synapses in the afferent pathways preceding the LGMD would be excited by such stimuli, and would habituate. This would render the MD system blind to small field movements which followed soon after the cessation of whole field movement, and would clearly be maladaptive. But in our experiments only rapid whole field stimuli produced postconvergence inhibition in the MD system (Fig. 3). Slow whole field movements produced few excitatory postsynaptic potentials (EPSPs) in the LGMD and did not appreciably decrease responsiveness to a subsequent small field stimulus, showing that such movements do not excite labile synapses in the pathways to the LGMD. There must, therefore, be a further inhibitory mechanism, which also suppresses the response to whole field stimuli, located peripherally in the optic lobe prior to the site of decrement. We demonstrated this inhibition (Fig. 4a); it suppressed the response to a small moving target, normally a very potent stimulus, and its effect was graded in proportion to the proximity of the large stimulating field to the small target. The dependence on spatial separation is typical of a classical lateral inhibitory network, in which the components of an array of similar afferent channels are mutually inhibitory in inverse proportion to their spatial separation. Figure 4b

Fig. 3 Postconvergence inhibition is produced only by rapid movements of large field stimuli. The entire field of the eye is occupied by a vertical array of black and white stripes. The upper trace (a) of each pair of records is an intracellular recording from the fan of the LGMD. The lower trace (b) of each pair is the output from a photocell which is also illuminated by the same stripes as produce the field. In A-C stripes are moved laterally across the visual field at velocities corresponding to 8, 20, and 70 light-dark or dark-light transitions per second at the retinal receptor. All speeds of movement tend to produce an increase in EPSPs; the faster movements increasingly produce large IPSPs as well. At eight transitions per second, no IPSPs are detectable.



shows that the lateral inhibition generated by a large moving stimulus not only largely suppressed the response to a simultaneously presented small target but also greatly reduced the rate and extent of habituation in response to the repeated movement of the small target. The dynamics of this lateral inhibition were such that during rapid whole field movements some excitation reached the LGMD, where EPSPs were generated; the peripheral lateral inhibition was supplemented by postconvergence inhibition acting in the LGMD. Such stimuli sometimes produce appreciable EPSPs in the LGMD, but these are prevented from initiating spikes by the summated IPSPs.

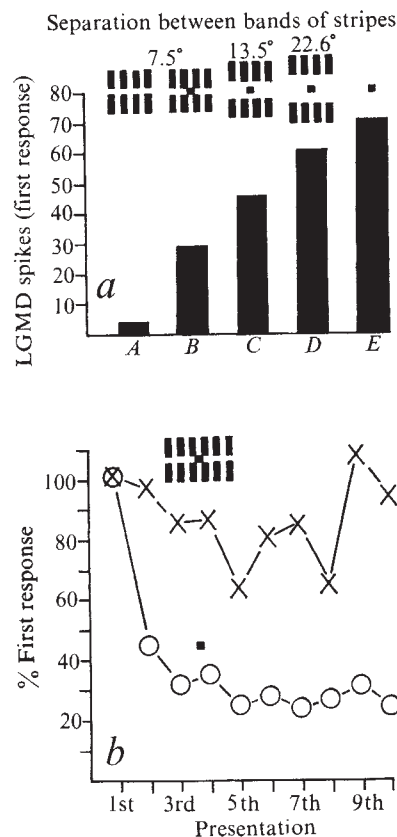


Fig. 4 Lateral inhibition of the response to a small target. *a*, The lateral movement of two bands of vertical stripes, which extend horizontally across the visual field, at a speed corresponding to seven transitions per second at the retinal cell, produces very few spikes in the LGMD (A). This speed of movement produces no postconvergence inhibition (compare Fig. 3). When a small black target is added to the stripes, the response is appreciably increased. The size of the response is a function of the proximity of the striped pattern to the small target (B-D) and the full response to the target is not seen until the stripes are totally removed (E). Trials were made at 2-min intervals in the order A-E; any habituation effects would therefore tend to counteract, rather than reinforce, the trend shown here. The dimensions on the diagram are in terms of the angle subtended at the eye. The two bands of stripes each subtend 22.6° vertically, and the period of the striped pattern is 15°. The small target subtends 7.5°. *b*, Lateral inhibition takes place more peripherally than habituation, and protects the labile synapses from depression during whole field movement. The response to repeated movements (interstimulus interval = 10 s) of the small target alone shows a rapid decrement from its original high level (○). When a large area of moving stripes is added to the stimulus in close proximity to the target, however, the response is not only depressed (see A) but also shows no significant decrement (×). Both curves are normalised for comparison.

Lateral inhibition is widespread in sensory systems of both vertebrates and invertebrates, and various functions have been suggested for it, such as contrast enhancement and an increase in the information content per action potential^{9,10}. In the MD system it has two other functions. First by preventing a response to whole or large field stimuli, it is the major factor in determining that the MD system responds preferentially to small-field stimuli and ignores visual displacements generated by the animal's own movements. Second, by acting prior to the site of decrement it protects the labile synapses from habituation, and therefore preserves the novelty detecting properties of the LGMD.

We believe protection from habituation to be a new and possibly general role for lateral inhibition, and it may prove to be important in other than visual systems. Protection from habituation is almost certainly a common device, because of the necessity of distinguishing sensory afference caused by the animal's own activities from that which is exogenous. For example, tactile afferent pathways are protected during the escape tailflip of the crayfish by an efferent control network derived from the motor output system which produces pre-synaptic inhibition^{11,12}. The protection mechanism we describe is derived from the afferent pathways and has the added property of being independent of the motor system; it therefore also operates in the stationary animal. This type of protection may also operate in the crayfish through inhibition generated by tactile afference (J. J. Wine and D. Kennedy, personal communication). Indeed, wherever an interneurone is found with a wide receptive field and a preference for localised and novel stimulation within that field, lateral inhibition between the pathways which feed it may be expected.

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MICHAEL O'SHEA
C. H. FRASER ROWELL

Department of Zoology,
University of California,
Berkeley, California 94720

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Inverse relationship between serum IgG concentrations and measures of intelligence in elderly persons

We have found a negative correlation between intelligence test scores and serum immunoglobulin concentrations in healthy subjects aged 45 yr and older. This finding adds a potentially important dimension in studies of older persons who exhibit declining intellectual function.

A longitudinal study of ageing in 268 elderly healthy volunteers was initiated in 1956 by the Duke University Center for the Study of Aging and Human Development. The composition of this subject sample was proportionate to the race, sex and socioeconomic composition of similarly aged persons in the community from which they were recruited (Durham, North Carolina). The Wechsler adult intelligence scale (WAIS) test was administered at the time of each visit to the centre. Assessment of immune function was begun in 1967 with measurements of serum concentrations of IgG, IgA and IgM. The method and reliability of these measurements has been detailed before¹.

Retrospective evaluation in 1973 revealed contemporaneous serum immunoglobulin levels and WAIS test scores for 97 persons (sample 1). Where more than one set of comparisons was available on any subject, the earliest comparison was selected for analysis. Sample 1 (mean age $\pm$ s.e.m. = 79.3 ± 0.5 yr; range 70-94) had a race and sex composition similar to the entire subject sample but had a higher mean WAIS test score. Both raw and scaled WAIS scores were evaluated; the results presented were independent of the type of score used. Serum immunoglobulin levels were $\log_e$ transformed before analysis; in normal populations the $\log_e$ values have a Gaussian distribution¹.

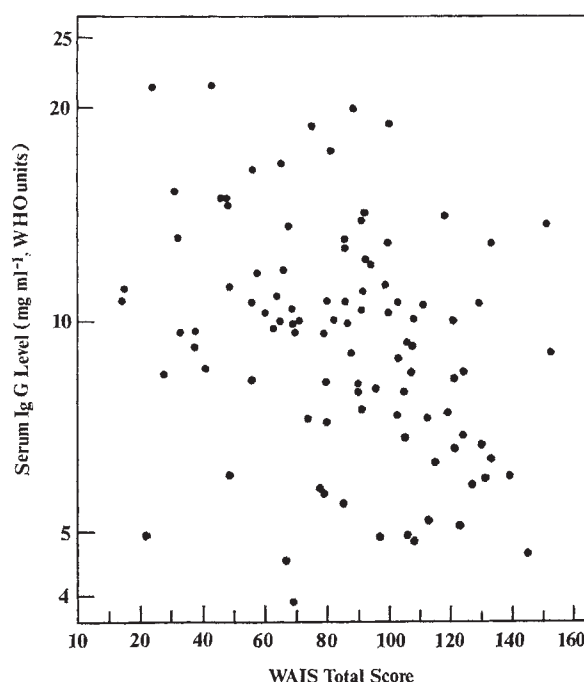


Fig. 1 Scattergram of WAIS total score (not age-corrected) and serum IgG level in 97 subjects from the Duke Longitudinal Study ($r = -0.332$, $P < 0.005$).

Evaluation of sample 1 revealed a significant negative correlation between serum IgG levels and WAIS scores ($r = -0.332$, $P < 0.005$) (Fig. 1); correlations for serum IgA ($r = 0.161$) and IgM ($r = 0.085$) were not significant (Table 1). This inverse relationship between the WAIS test and IgG was detected in all race and sex subgroups. Although only four subtests are presented in Table 1, all 11 WAIS subtests scores were inversely related to serum IgG level. In sample 1, WAIS score and age were correlated -0.157 ; IgG and age, were correlated 0.110 . To evaluate the relationship between the WAIS scores and serum IgG levels unencumbered by the effects of age, race and sex, a stepwise multiple regression analysis was performed with total WAIS score as the dependent variable. Even after age, race and sex were forced into the regression equation, IgG accounted for a significant portion of the WAIS variability (partial $r = -0.296$, increase in multiple $R^2 = 0.082$, $P < 0.001$). To exclude the possibility that other sources of variation, such as the effects of repeated WAIS testing, disease and terminal illness, might account for the inverse relationship, regression analysis was also performed with the initial test scores of subjects who had no clinical evidence of illness and did not die within 2 yr of the IgG determination ($n = 67$, mean age = 66.8 ± 0.6 yr). The IgG level still made a significant contribution to the regression equation (partial $r = -0.323$, increase in multiple $R^2 = 0.104$, $P < 0.005$). This suggests the observed relationship was not dependent on training effects or health status.

The negative relationship was reproducible. A second

Table 1 Correlations between WAIS scores and serum immunoglobulin level in two samples of older persons

	Sample 1*			Sample 2†		
	IgG	IgA	IgM	IgG	IgA	IgM
WAIS subtests‡						
Information	-0.363	0.183	0.061	0.018	0.006	0.006
Vocabulary	-0.319	0.120	0.065	-0.081	-0.065	-0.016
Digit symbol	-0.413	0.059	0.165	-0.098	-0.150	0.025
Picture arrangement	-0.217	0.152	0.045	-0.096	-0.100	0.026
Average scaled subtests§	—	—	—	-0.109	-0.121	0.027
Total WAIS score¶	-0.322	0.161	0.085	—	—	—

* $n = 97$, mean age $\pm$ s.e.m. = 79.3 ± 0.5 yr.

† $n = 468$, mean age $\pm$ s.e.m. = 58.5 ± 0.3 yr.

‡ All 11 WAIS subtests were administered to sample 1, but only the four in the table were administered to sample 2. The four subtests were selected because the previous study had demonstrated that the subtests, information and vocabulary, showed the most stability with increasing age, and the subtests, digit symbol and picture arrangement showed the greatest decline. The seven subtests administered to sample 1 but omitted from this table were all negatively correlated with serum IgG level.

§ Because an overall value such as total WAIS score was not available for sample 2, the average of the scaled scores for the four subtests was used.

¶ This score was not age-corrected.

longitudinal study was initiated at Duke University in 1967 consisting of 502 white persons aged 46–71 yr. These subjects represented a broad range of the middle and upper socioeconomic classes of Durham. On the basis of the experience with the first longitudinal study, only four WAIS subtests were administered. Information and vocabulary subtests were selected because they had shown the least change with age. Digit symbol and picture arrangement were selected because they had shown the greatest change with age. Sample 2 consisted of 468 persons (mean age = 58.5 ± 0.3) on whom both WAIS scores and immunoglobulin levels were available from the initial round of testing. In this younger, racially homogeneous sample, the correlation between IgG level and an average of the four scaled subtest scores was negative ($r = -0.109$, $P < 0.02$). Seven out of ten (sex-by-5 yr age) subgroups in sample 2 showed the same inverse relationship. Three of the four subtests exhibited a negative correlation with serum IgG level (Table 1). A similar relationship was detected in subject sample 2 between serum IgA and WAIS scores (Table 1). After deletion of age and sex effects, stepwise multiple regression analyses revealed that either IgG or IgA level made a significant, but not independent, contribution to the regression equation predicting each subject's averaged scaled subtest scores (for IgG, partial $r = -0.086$, increase in multiple $R^2 = 0.012$, $P < 0.025$). This association between serum IgA and WAIS test scores could be the result of the relationship between IgG and IgA previously reported in younger persons². IgG and IgA were significantly correlated in sample 2 ($r = 0.26$, $P < 0.001$), but not in the older sample 1 ($r = 0.13$).

The variability in WAIS test scores associated with IgG levels in both samples was small but significant. The magnitude of this association could be a consequence of many other sources of variation; alternatively it may reflect a major influence present in only some members of each sample. Although a precise mechanism cannot be identified from our data, the sevenfold increase in variability accounted for in the older subject sample is consistent with some consequence of biological age. Several testable ageing-dependent hypotheses can be advanced to explain the observed relationship. First, anti-brain antibodies increase with age³ and might have a pathogenic role in cognitive decline. Second, age-related decline in a third physiological system might account for decreased intellectual performance and elevated IgG levels. Finally, an effect of selective mortality cannot be excluded. Two lines of evidence support this last possibility. First, individuals in both samples who died within 5 yr of the time of measurement (27 subjects in sample 1 and 28 subjects in sample 2) had lower WAIS scores and IgG levels than their surviving cohorts. Second,

patients with senile dementia, which is associated with early death⁴, have significantly lower serum IgG levels than age matched controls (R. Green and C. E. Buckley, unpublished). A shortened life expectancy in persons with low IgG levels and low WAIS scores could contribute to the inverse relationship between measures of immune and intellectual performance detected in older persons.

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JEFFREY M. ROSEMAN

Center for the Study of Aging and Human Development,

C. EDWARD BUCKLEY III

Department of Medicine,
Duke University Medical Center,
Durham, North Carolina 27710

Received July 1, 1974; revised January 24, 1975.

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In vivo evaluation of microcirculation by coherent light scattering

THE microcirculation plays a central role in the regulation of the metabolic, haemodynamic and thermal state of the individual. Its physiological state varies over both long and short time periods and reflects the state of health of the individual; in particular it is the ultimate arbiter of the adequacy of tissue perfusion in the presence of vascular disease or injury. For these reasons it is of interest to both the physiologist and the clinician to be able to monitor the state of flow and distribution of flow in the compartments of the microvasculature rapidly, conveniently and with minimal interference with the many local and central reflex controls. At present such measurements depend on direct observation, plethysmography thermal or radioisotope techniques¹, which are slow or cumbersome or grossly disturb the normal state of the subject. We report here the first stages in development of a noninvasive method for acquiring such information which has a rapid response and the potential ability to distinguish flow in the different microvascular compartments by the differences in flow velocities.

When coherent light is scattered from a complex medium which is in internal motion, the spectral line is broadened by the Doppler effect in a manner characteristic of the velocity distribution of the motion. By heterodyning or homodyning the light on a square-law photodetector, this bandwidth may be brought to audio frequencies and examined with an electronic spectrum analyser². Information so obtained has been used in biology for measurement of diffusion coefficients of macromolecules³, motility of bacteria^{4,5}, blood flow in retinal arteries⁶ and viability of sperm⁷.

Although the vascular bed of, for example, the skin is "visible" from the surface, in the sense that it imparts colour to the skin, light which reaches the blood and returns will generally suffer multiple scatterings during its diffusion through tissues. At present this precludes a quantitative theoretical calculation of the Doppler spectrum, which must instead be calibrated empirically against conventional physiological measures, using the predictions of idealised theoretical models as a guide.

As an example of the latter, we might imagine that photons wander randomly through densely diffusing stationary tissues, encountering one or more red blood cells moving in random directions with a speed distribution $U(v)$. The distribution $P_1(\Omega)$ of Doppler shifts of those photons which encounter one

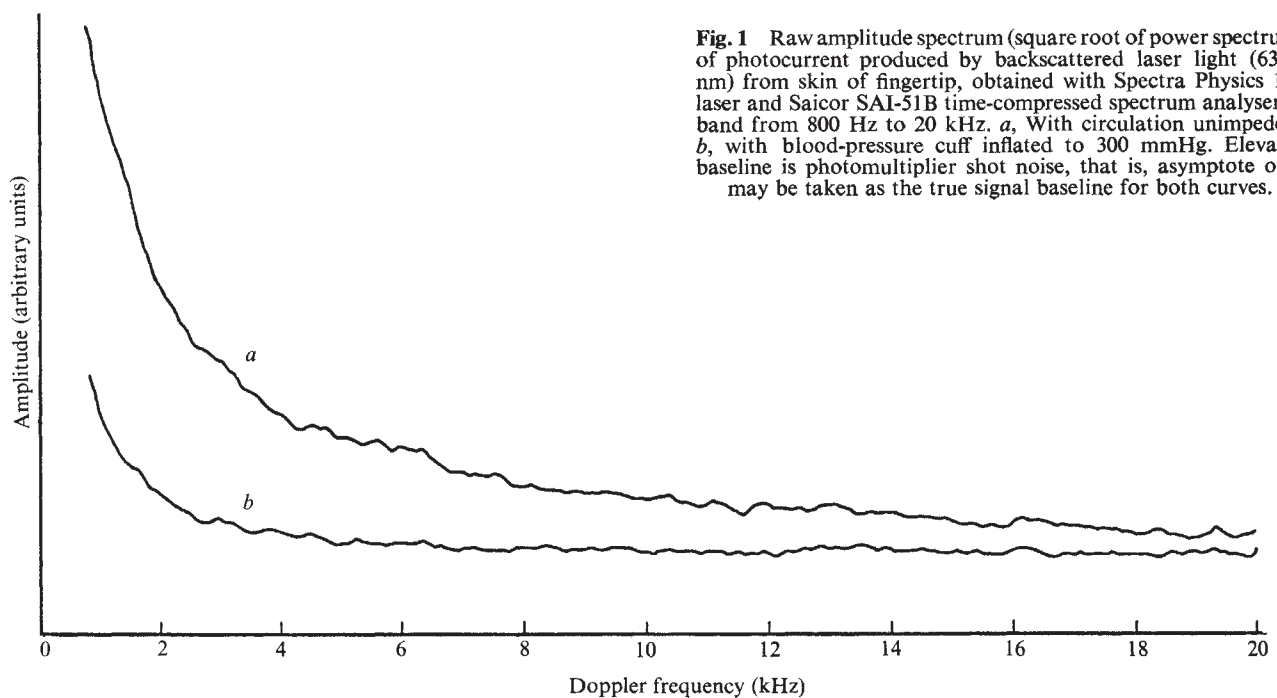


Fig. 1 Raw amplitude spectrum (square root of power spectrum) of photocurrent produced by backscattered laser light (632.8 nm) from skin of fingertip, obtained with Spectra Physics 124 laser and Saicor SAI-51B time-compressed spectrum analyser in band from 800 Hz to 20 kHz. *a*, With circulation unimpeded; *b*, with blood-pressure cuff inflated to 300 mmHg. Elevated baseline is photomultiplier shot noise, that is, asymptote of *b* may be taken as the true signal baseline for both curves.

red cell may be found from the basic Doppler formula $\Omega = \mathbf{K} \cdot \mathbf{v}$ by averaging over all the random directions involved; after some manipulation of integral identities it may be shown that

$$v^2(d^2P_1(2v/\lambda))/dv^2 = \lambda U(v)/2$$

where λ is the wavelength of the light. The higher order multiple red cell encounters, $P_n(\Omega)$ are the self-convolutions of $P_1(\Omega)$, since the photon suffers the sum of a number of independent random Doppler shifts. From arguments of this sort it may be shown that: (1) in principle it is possible to recover the cell speed distribution $U(v)$ from the measured spectrum; (2) the spectrum is expected to be a monotonically decreasing function of frequency, higher frequencies corresponding to faster flow speeds, and possibly possessing an integrable singularity at $\Omega = 0$; (3) the scattered light will be depolarised, with two statistically independent polarisation components each having Gaussian statistics; (4) for a random flow field with an r.m.s. velocity of 1 cm s^{-1} the r.m.s. bandwidth of 632.8 nm light will be 12,903 Hz times the square root of the mean number of red cell encounters (plus small corrections for the shape of red cells and refractive index).

These conclusions are, of course, based on idealised assumptions

and should be taken only as a guide to the interpretation of empirical spectra.

We have undertaken preliminary experiments to determine the feasibility of this method. Light from a 15 mW HeNe laser illuminated a 1 mm area of skin of a fingertip. The backscattered light was sensed through a pinhole by a photomultiplier. The homodyne photocurrent which resulted was passed through a variable bandpass filter to a time compression digital spectrum analyser. To evaluate the possibility of following rapidly the dynamics of the circulation, the signal within a particular passband was rectified and displayed on a chart recorder as a function of time.

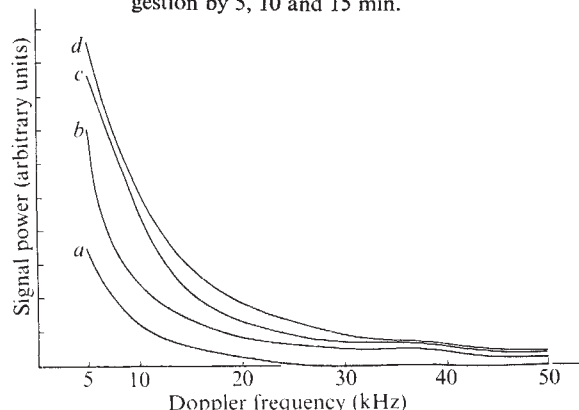
Figure 1 shows the spectrum before and after occlusion of the brachial artery with a blood-pressure cuff, demonstrating that the Doppler signal is indeed caused by blood flow. The bulk of the flow signal power is actually in the band below 1 kHz but that region is more subject to artefacts resulting from tissue motion in the crude preliminary apparatus.

To demonstrate the feasibility of monitoring pharmacological effects on the microvasculature, a known vasodilator, in this case a small amount of ethanol, was administered. Figure 2 shows successive spectra. The frequency band shown corresponds to speeds found only in arterioles, venules, and shunts. The result is consistent with increased flow in large vessels and increased maximal flow velocity.

Figure 3 shows the variation over time of the signal power in the 10–12 kHz band as a blood-pressure cuff is rapidly inflated above the systolic pressure and then slowly let down. The initial decay is the time required for large arteries to empty before the microvascular flow stagnates. When the cuff is held between systolic and diastolic pressures until the veins have filled, the flow becomes highly pulsatile. In this time-resolved mode it was observed that fingertip flow is quite labile in response to room temperature, posture, respiratory pattern and emotional stimulation of the subject. We know of no comparable method of demonstrating these rapid microvascular reflexes.

Laser Doppler spectroscopy therefore offers a promising tool for examination of the physiological state of the microcirculation rapidly and non-invasively. Because it provides information about the flow velocity distribution, it has the potential ability to analyse the distribution of flow in the different microvascular compartments. Applications in vascular physiology, clinical evaluation of vascular disease and assessment of the viability of burns and grafts are foreseen. We would like to thank Dr Ralph

Fig. 2 Power spectrum of Doppler flow signal from fingertip at intervals following ingestion of 30 ml of ethanol. Arbitrary units. Bandpass 5–50 kHz. Data smoothed by neighbouring point averaging corrected for shot noise and bandpass filter response. *a*, Control before ethanol administration; *b*, *c* and *d* follow ingestion by 5, 10 and 15 min.



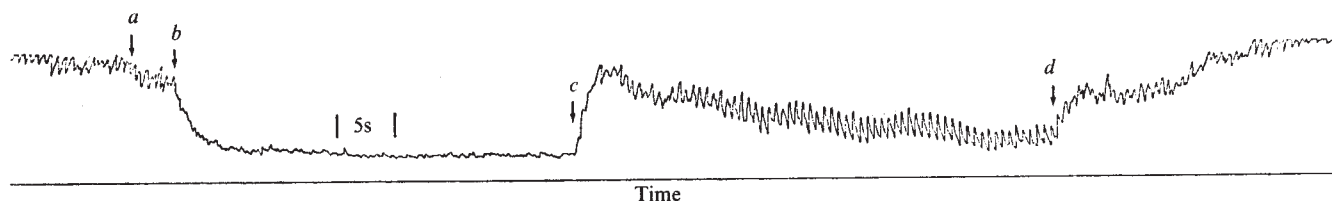


Fig. 3 Amplitude of Doppler flow signal (including shot noise baseline) in the band 10–12 kHz as a function of time during inflation of a blood-pressure cuff followed by its slow release. Point *a* is beginning of inflation. At *b* the cuff exceeds the systolic pressure. Cuff pressure was held above systolic for 20 s and then slowly released. At *c* the cuff pressure dropped below systolic; at *d* cuff pressure fell below diastolic. By the end of the chart cuff pressure was entirely released. Amplitude scale arbitrary.

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M. D. STERN

Laboratory of Technical Development,
National Heart and Lung Institute,
National Institutes of Health,
Bethesda, Maryland 20014

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Alterations in behaviour and catecholamine biosynthesis induced by lithium

THERE is general agreement that lithium (Li) impairs central catecholamine transmission, although the underlying mechanism is subject to debate^{1–4}. More ambiguous is the literature describing the behavioural effects of Li in experimental animals. Although considerable evidence indicates that Li suppresses various behaviours^{5–10}, there are reports that this effect is only apparent after drug-induced stimulation^{11–14}. Results are also contradictory with respect to the behavioural effects of long term Li administration, some studies reporting continued or even exaggerated suppression with repeated administration^{15–18}, while others describe development of tolerance to the Li-induced behavioural suppression^{12,13}.

Lithium is well established as a treatment for mania and a prophylactic agent for prevention of recurrent mood disorders^{19–21}. Thus, the elucidation of its behavioural and neurochemical effects may provide some insight into the aetiology of naturally occurring mood disorders. Since some degree of long term treatment with Li is, however, usually required before clinical improvement is apparent, it is essential to evaluate potential clinically relevant effects in chronically treated animals. In our study of the behavioural and neurochemical correlates of long term Li administration, we have found that Li suppresses both spontaneous and amphetamine-induced behaviour in rats; that tolerance develops to this suppression, and that the emergence of tolerance is associated with increased tyrosine hydroxylase activity in the substantia nigra and caudate-putamen of rats.

Male rats (350–375 g, Carworth Farms) were housed in groups of four and maintained on a 12-h light–dark cycle and allowed free access to food and water. Locomotion was monitored automatically in sound-attenuated chambers^{22,23}.

All rats received nine daily injections, the last immediately before testing in the chambers. The group on a regime of long term treatment received eight daily injections of LiCl (1.5 meq kg⁻¹) and a single injection of saline before testing. Two

acute groups were injected with Li, either 24 h or immediately before testing. The control group received nine injections of saline. Injections were given subcutaneously at the same time each day in volumes of approximately 3 ml in order to minimise local irritation produced by Li.

After the final pretreatment injections, animals were placed in the chambers and their spontaneous activity was monitored for 2 h. Then, all groups received an injection of *d*-amphetamine sulphate (0.5 mg kg⁻¹, intraperitoneally as free base). Behaviour was monitored for an additional 2 h, at which time the behavioural response to this dose of amphetamine had subsided^{22,23}.

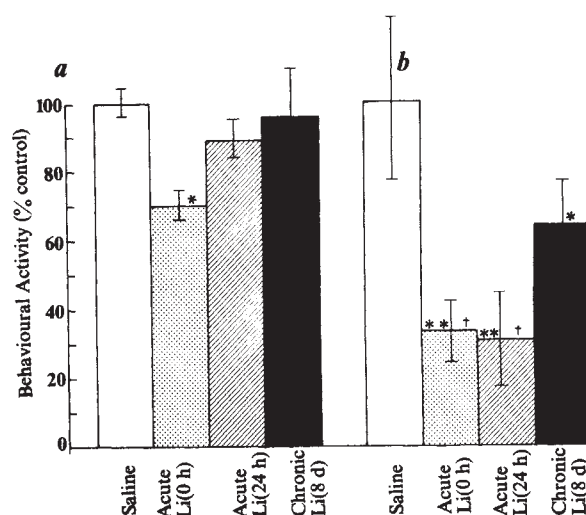


Fig. 1 Effect of acute and chronic Li administration on spontaneous locomotor activity (*a*) and on *d*-amphetamine-induced behavioural excitation (*b*). Single injection of Li (1.5 meq kg⁻¹), subcutaneously only reduced spontaneous activity in the 0-h acute group. Excitation produced by amphetamine (0.5 mg kg⁻¹, intraperitoneally), however, was depressed in both acute groups and, to a lesser extent, in the 8-d Li group.

*Significantly less than saline pretreated controls, $P < 0.02$.

†Significantly less than chronic Li group, $P < 0.05$.

**Significantly less than saline pretreated controls, $P < 0.001$.

During the first 30 min after initial exposure in the chambers, only the 0-h acute group showed a decrease in activity compared with saline controls ($P < 0.02$; Fig. 1). During the second-hour interval before injection of amphetamine, all groups remained relatively inactive. Injection of amphetamine produced a moderate increase in locomotion for the first 30 min after injection (approximately 100 cross-overs for the saline pretreated group). Acute pretreatment with Li significantly suppressed the amphetamine-induced activity to 30–40% of saline control levels ($P < 0.01$) for up to 24 h after injection of Li. Tolerance to the suppressive effects, however, seems to have occurred by 8 d, for the animals repeatedly injected with Li exhibited significantly more amphetamine-induced activity than did either of the acute groups ($P < 0.05$), but less than that of the saline control group ($P < 0.02$).

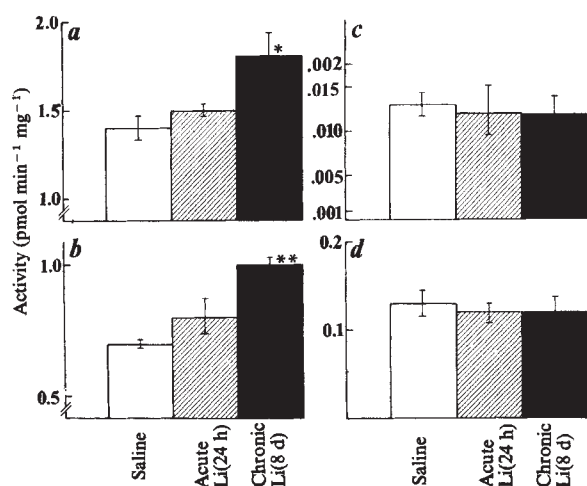


Fig. 2 Effect of acute and chronic Li administration on tyrosine hydroxylase activity in the cell body and nerve ending regions of the nigro-striatal pathway (substantia nigra (b) and caudate-putamen (a), respectively) and in the dorsal noradrenaline pathway (locus coeruleus (d) hippocampus-cortex (c), respectively). Eight-day Li treatment resulted in an augmentation in tyrosine hydroxylase activity only in the substantia nigra and caudate-putamen.

* $P < 0.05$.

† $P < 0.001$.

To examine the possible neurochemical correlates for these behavioural effects, rats comparable with those used for the behavioural experiments were injected with either saline or Li for 8 d, or with saline for 7 d and with Li on the eighth day. All rats were decapitated 24 h after the last injection, their brains were removed quickly and four discrete regions were dissected using a brain slicing device²⁴. The regions were homogenised individually and examined for tyrosine hydroxylase activity by a modification of the Nagatsu procedure^{25,26}. The areas examined included the cell body and nerve ending regions of the dopaminergic nigro-striatal pathway (substantia nigra and caudate-putamen, respectively), and the cell body and nerve ending regions of the dorsal noradrenergic pathway (locus coeruleus and hippocampus-cortex, respectively).

Alterations in tyrosine hydroxylase activity were found only in the dopaminergic system (Fig. 2). Acute Li produced a slight although insignificant increase in tyrosine hydroxylase activity. But 24 h after eight daily injections of Li, the enzyme activity in both substantia nigra and caudate-putamen had increased substantially (30 and 40% greater than corresponding controls), thus suggesting that the nigro-striatal dopamine pathway plays a role in the development of tolerance to the behavioural suppression induced by Li. That is, an increased availability of dopamine, consequent to the elevation of tyrosine hydroxylase activity, might partially overcome the impaired transmission produced by Li.

The failure to obtain enzyme changes in the noradrenaline regions examined seems to conflict with other reports of Li-induced increases in the turnover rate of brain noradrenaline^{16,27-29}. Most of these results are, however, based on whole brain assessment of turnover, while our study was restricted to the dorsal pathway. It is conceivable, therefore, that the Li-induced alterations in turnover of noradrenaline are limited to the ventral or other brain noradrenaline systems. The lack of a response in the dorsal noradrenaline pathway does not indicate that in this system tyrosine hydroxylase activity is refractory to change, since we have found that either chronic reserpine or desmethylimipramine administration produces marked alterations in the activity of tyrosine hydroxylase in the locus coeruleus and hippocampus-cortex³⁰.

With respect to brain dopamine pathways, Ho *et al.*³¹ report no alterations in turnover in four brain regions after several

weeks of Li treatment. Friedman and Gershon³², however, found a substantial decrease in dopamine biosynthesis in striatal brain slices after 14 daily injections of Li. Several differences in procedure may account for the discrepancy between our results and those of Friedman and Gershon, not the least of which is the large difference in time between last injection and biochemical determination (that is, 1 h compared with 24 h).

Whether or not continued chronic Li treatment would result in complete tolerance to its suppressive effects remains to be determined; however, if complete tolerance is found to occur, then the action of Li on amphetamine-induced behavioural hyperactivity would seem to be an inappropriate model for its clinical effects. Irrespective of the clinical implications, however, the neurochemical events underlying the tolerance development to Li-induced behavioural suppression are of considerable theoretical interest. We have shown before that prolonged impairment of central catecholamine transmission, produced by drugs or other treatments, results in an increase of tyrosine hydroxylase activity³³⁻³⁵. The converse relationship is obtained when synaptic transmission is facilitated for relatively long periods³⁰. Thus, our finding that an augmentation of tyrosine hydroxylase activity is produced by Li is consistent with these findings. Such an increase in catecholamine biosynthetic capacity may represent a compensatory response to the Li-induced impairment of catecholamine transmission which partially counteracts the suppressive effects of Li on behaviour.

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DAVID S. SEGAL
MAUREEN CALLAGHAN
ARNOLD J. MANDELL

Department of Psychiatry,
University of California, San Diego,
Medical School,
La Jolla,
California 92037

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Lipopolysaccharide induces C-type virus in short term cultures of BALB/c spleen cells

In certain strains of mice, such as AKR, C-type viruses occur throughout life and can readily be detected by infectivity assays^{1,2}. In other seemingly virus-free strains, C-type viruses can be activated *in vivo* by X irradiation^{3,4}, chemical carcinogens⁵, graft-versus-host reactions⁶ and *in vitro* by long term cultivation⁷, by X rays⁸, halogenated pyrimidine analogues^{9,10} and mixed lymphocyte reactions⁸. We report here that lipopolysaccharide, a B-cell mitogen¹⁸, induces C-type virus in short term cultures of BALB/c spleen cells.

During the first 24 h, spleen cell cultures from 6–8 week old male BALB/c mice (Bomholdgard, Denmark) contained lipopolysaccharide W, *E. coli* 0111:B4 (LPS, Difco, 16 $\mu\text{g ml}^{-1}$) or concanavalin A (con A, Calbiochem, 4 $\mu\text{g ml}^{-1}$) or phytohaemagglutinin-P (PHA, Difco, 10 $\mu\text{g ml}^{-1}$) or no mitogen. Pellets obtained from the pooled day 3 and day 4 supernatants of four groups (LPS, con A, PHA and control), each originating from 50 cultures (1 ml) were assayed for reverse transcriptase activity¹² with poly(A)_n·poly(dT)₁₂₋₁₈ as template primer. Figure 1 shows that LPS-treated culture supernatants contained enzyme activity which was linear for about 60 min, while in control cultures or in con A- and PHA-stimulated cultures no activity was found. Enzyme induction could be reproduced using a highly purified LPS from *Salmonella abortus equi*.

To characterise further this activity, 25 combined LPS-treated 1-ml cultures were analysed following isopycnic centrifugation on a 15–60% sucrose gradient (Fig. 2). A peak of activity was

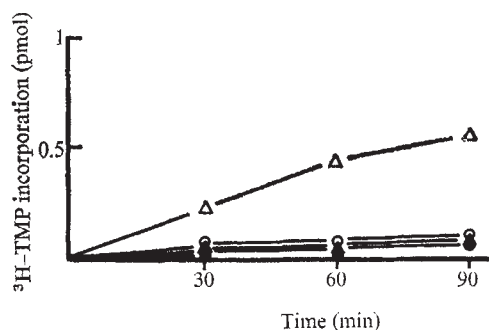


Fig. 1 Reverse transcriptase assay in samples prepared from concentrated supernatants from cell cultures stimulated with LPS (Δ), con A ($\blacktriangle$), PHA ($\bullet$), or control ($\circ$). Reaction mixtures (100 μl) contained: 20 μl sample, 40 mM Tris, pH 7.9, 60 mM KCl, 1 mM dithiothreitol, 1.5 mM Mn(II)-acetate, 0.2 mM EDTA, 0.1% Nonidet P-40, 20 $\mu\text{g ml}^{-1}$ poly(A)_n·poly(dT)₁₂₋₁₈ (poly(A)_n (Miles) and dT (Collaborative Research) were annealed in equimolar amounts), 10 μM ^3H -TTP (Amersham, 7,500 d.p.m. pmol⁻¹). Incubation at 30 °C was stopped at the indicated times by the addition of an excess of 10% trichloroacetic acid (TCA) containing 0.02 M sodium pyrophosphate. Following 30 min incubation in ice, acid-precipitable radioactivity was washed on a GF/C glass fibre filter (Whatman) with cold 5% TCA and processed for liquid scintillation counting using standard procedures. Reaction mixtures containing no virus were included for background determination which was subtracted from the experimental values. Spleen cell cultures were prepared as described previously¹¹. A 1 ml culture contained 2×10^6 viable nucleated cells in loosely capped disposable 12 $\times$ 75 mm tubes (Falcon). Incubation was at 37 °C in CO₂ in air (8%). Culture medium RPMI 1640 (Microbiological Associates) containing 8% foetal bovine serum (Rehatain, Reheis), penicillin (50 IU ml⁻¹, Hoechst) and streptomycin (50 $\mu\text{g ml}^{-1}$, Novo) was changed every 24 h. The removed medium from days 2–4 was centrifuged (1,000g for 10 min) and kept at –20 °C until further examination. Then, 1 ml samples were pooled from corresponding cultures after thawing at 30 °C, centrifuged (35,000g for 60 min) at 4 °C and resuspended in 1/50 of the original volume in buffer (0.01 M Tris, 20 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 50% (v/v) glycerol, pH 7.9).

observed at 1.16 g cm⁻³, the density characteristic for C-type viruses.

Some cellular enzymes are known to polymerise thymidine monophosphate using poly(A)_n·poly(dT)₁₂₋₁₈ as template primer, but they work more efficiently with poly(dA)_n·poly(dT)₁₂₋₁₈ (refs 13–15). Moreover, terminal nucleotidyl transferase from thymus cells can be stimulated by dT₁₂₋₁₈ (ref. 16). To distinguish whether the LPS-induced

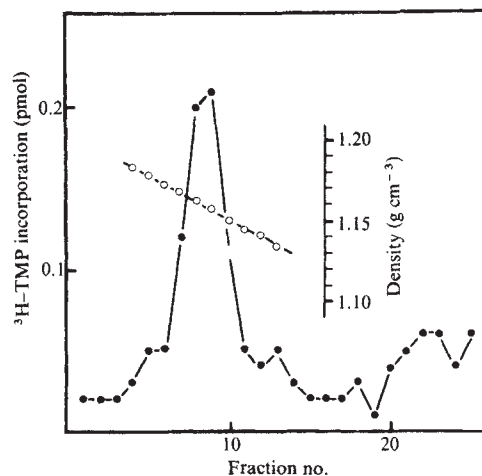


Fig. 2 Sucrose density gradient centrifugation. A pellet prepared from pooled day 2 to 4 supernatants of LPS-stimulated cultures was resuspended in phosphate buffered saline, pH 7.4, layered over a 15–60% sucrose gradient and centrifuged in a Beckman SW65 rotor at 35,000 r.p.m. for 16 h at 4 °C. Three drop fractions were collected from the bottom and 50 μl per fraction were analysed for reverse transcriptase activity (90 min assay). For conditions see Fig. 1. Density determinations of selected fractions were performed with an Abbé refractometer.

enzyme activity represents C-type viral reverse transcriptase or cellular enzymes, poly(A)_n·poly(dT)₁₂₋₁₈, poly(dA)_n·poly(dT)₁₂₋₁₈ and poly(dT)₁₂₋₁₈ were compared for their capacity to stimulate the enzyme (Table 1). Sucrose gradient purified Rauscher leukaemia virus (RLV) and a spleen cell homogenate were included as controls. LPS-induced activity as well as reverse transcriptase from RLV showed a strong preference for poly(A)_n·poly(dT)₁₂₋₁₈ over poly(dA)_n·poly(dT)₁₂₋₁₈ but no detectable activity with poly(dT)₁₂₋₁₈. The cellular enzymes preferred poly(dA)_n·poly(dT)₁₂₋₁₈ and were also stimulated by poly(dT)₁₂₋₁₈. The presence of C-type particles in LPS-stimulated cultures was verified using electron microscopy

Table 1 Template-primer requirements for enzyme activity

Source of enzyme	Template-primer		
	A _n ·dT ₁₂₋₁₈	dA _n ·dT ₁₂₋₁₈ *	dT ₁₂₋₁₈ †
Supernatant of LPS-stimulated spleen cells‡	0.79	0.08	0.05
Rauscher leukaemia virus	9.87	0.06	<0.01
Supernatant of normal spleen cell homogenate§	0.14	0.83	0.07

Same assay conditions as described in Fig. 1. Incubation time was 90 min. Mean values of triplicate determinations are expressed in pmol ^3H -thymidine-monophosphate incorporated per 20 μl concentrated supernatant.

* poly(A)_n·poly(dT)₁₂₋₁₈ of standard reaction mixture replaced by poly(dA)_n·poly(dT)₁₂₋₁₈.

† poly(A)_n·poly(dT)₁₂₋₁₈ of standard reaction mixture replaced by poly(dT)₁₂₋₁₈.

‡ Pooled supernatants harvested on days 3 and 4.

§ BALB/c spleen cells were homogenised by hand in normal saline and cleared by centrifugation.

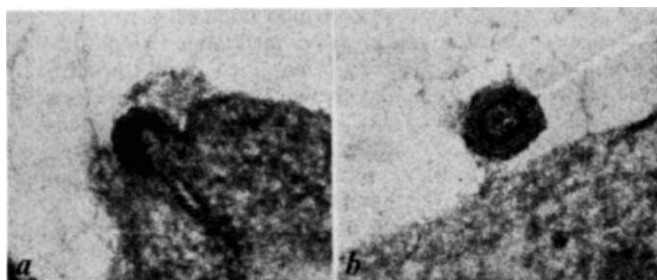


Fig. 3 Electron micrographs of C-type virus particles in 3-day BALB/c mouse spleen cultures induced by LPS. Sections from cells double-fixed in suspension with glutaraldehyde and osmium tetroxide ($\times 120,000$). a, Budding particles; b, extracellular mature particles.

(Fig. 3). Typical budding as well as extracellular virus could be detected.

It is surprising that an ubiquitous agent such as LPS, a constituent of Gram-negative bacteria, physiologically occurring in the intestinal flora, induces C-type virus. Evidence that induction of endogenous C-type virus may be quite frequent *in vivo* was reported by Aaronson and Stephenson¹⁷ who found that most mouse strains tested had high antibody titres against endogenous xenotropic virus. The relationship between LPS-induced virus, endogenous mouse-tropic virus and xenotropic virus should be investigated further. It will be interesting to see whether LPS also induces C-type viruses in other species, including man.

Purified LPS from *Salmonella* was provided by Dr Ch. Galanos, Max-Planck-Institut für Immunobiologie, Freiburg i.Br. Dr E. Suter carried out the electron microscopic study.

CHRISTOPH MORONI

Friedrich Miescher-Institut,
Basel, Switzerland

GEBHARD SCHUMANN

Biological Research Laboratories,
Pharmaceutical Division,
CIBA-GEIGY Limited, Basel, Switzerland

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Sudden appearance of IgG plaque-forming cells

EXPERIMENTS described here show that a large number of indirect, presumably IgG, plaque-forming cells (PFC) appear within a few hours during the initial stages of the IgG response of BALB/c mice to sheep red cells. When washed spleen cells

are placed *in vitro* over the appropriate period indirect PFC develop as well as *in vivo*, but a few hours earlier.

These experiments began when it was found that the count of indirect PFC (using the technique of Cunningham *et al.*¹) rose from an undetectable level on the fourth day after immunisation (day 4), to the peak value (2×10^5 to 4×10^5 per spleen) by early on day 5. We followed events during day 4 in

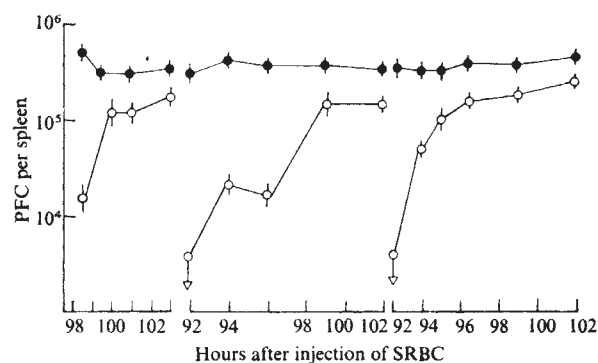
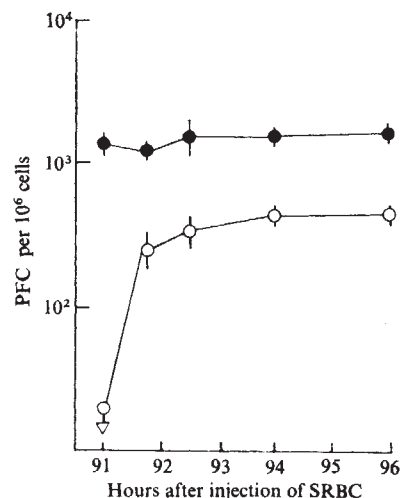


Fig. 1 Groups of 6 BALB/c female mice received 10^8 sheep red blood cells (SRBC) intravenously and PFC were assayed at intervals thereafter, by the method of Cunningham *et al.*¹, but incubating for 30 min only. Indirect PFC were developed by a rabbit serum against mouse IgG. This was raised using purified mouse IgG and, on immunoelectrophoresis against whole mouse serum, showed only a single line, reacting with IgG. It slightly inhibits PFC on day 2, when one expects the majority of PFC to be IgM (ref. 3) and considerably increases the count from day 5 onwards when the majority of PFC are IgG. The day-to-day levels of PFC developed by this serum follow closely those reported by other workers². Points are geometric means. Bars indicate 1 s.e. ●, Direct PFC; ○, Indirect PFC; circle joined to triangle, value less than lowest level detectable (4,000 per spleen).

detail and, in repeated experiments, found that the count had risen to around 10^5 within 8–9 h. When we followed the count at intervals of approximately 2 h, we found a sharp rise within the initial 2–4 h (Fig. 1). These figures are means from groups of 6 mice and, as it is unlikely that the rises were simultaneous, the increase in an individual mouse could have been even more rapid.

The same rise in IgG PFC was seen when washed spleen cells were incubated *in vitro* over the appropriate period. The best

Fig. 2 Spleens were removed 90 h after SRBC injection. A pool of washed cells was prepared in RPMI 1640 containing 5% foetal calf serum. 1 ml containing 3×10^7 nucleated cells was placed in each of several polystyrene tubes (12×75 mm), which were gassed with CO_2 -air (5:95) and rotated on a blood cell suspension mixer at 37°C . At the times shown, 0.05 ml was removed for PFC assay, and the tubes regassed and replaced. Tubes which had not been opened during the experiment gave the same counts at 96 h as those which had been sampled repeatedly.



results were obtained in tubes rocked or rotated so that the cells remained in suspension: in these conditions few cells became adherent. One of these experiments is shown in Fig. 2; during a 1.5-h incubation (2.5 h after spleen removal) a large number of IgG PFC appeared and the ratio of indirect to direct PFC is similar to what is found *in vivo* (compare Fig. 1). Experiments of the type illustrated in Fig. 3 show that populations placed in culture can develop IgG PFC earlier than they would *in vivo*. For instance, population B, removed from mice at 86 h has developed 300 IgG PFC per 10^6 cells (equivalent to 3×10^4 or more per spleen) by 90 h, while population D, when removed from the same group of mice at 91 h had no demonstrable IgG PFC.

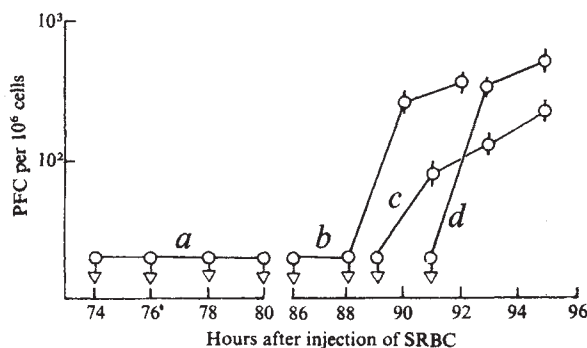


Fig. 3 As Fig. 2 but cells removed at different times after injection of SRBC. Only indirect (IgG) PFC are shown, to avoid complicating the figure. Counts of direct (IgM) PFC varied no more than in Fig. 2; and had the following mean values: a, 334; b, 1,406; c, 957; d, 1,439 PFC per 10^6 cells.

Our results suggest the following sequence of events in these mice. Between 74 and 86 h the spleen develops the ability to generate rapidly a large number of IgG PFC. The results obtained *in vitro* show that this process (the generation of IgG PFC) does not require a structured microenvironment, nor continued humoral influences, nor perhaps adherent cells. From 86 h (when IgG PFC can appear *in vitro*), the process is suppressed *in vivo* until around 92 h when a large number of PFC appear suddenly.

The development of large numbers of IgG PFC within periods of 12–24 h has been observed in several other strains of mice^{2–4}, hence the events we have described may occur more widely.

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I. E. ADDISON

Department of Experimental Pathology,
St. Mary's Hospital Medical School,
London W2 1PG, UK

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***In vitro* ageing and evolution of immunocompetence in long term normal lymphoid cell cultures**

CULTURE of dissociated lymphoid cells *in vitro* enables the description of various characteristics of an immune response as manifested under defined conditions—but not the time course of evolution. Normal lymphoid cell cultures are usually

short term, those of spleen or lymph node cells from common laboratory animals rarely surviving longer than 12–14 d. Lymphoblastoid or plasmacytoma lines, established in continuous cultures originated from cancerous or Epstein-Barr virus-bearing tissues, seem to have lost their capacity for responding to specific antigens. We have shown^{1,2} that calf lymphoid cells can be maintained in more prolonged cultures (up to three months) without losing their immunocompetence. We report results obtained using dissociated calf lymph node cell cultures surviving for more than 2 yr.

Culture methods were as described previously¹: briefly, lymph nodes from 3–4-month-old animals (local slaughterhouse) within 5 min after slaughtering, were freed from surrounding tissue, quickly swabbed with ethanol, and brought to the laboratory in sterile culture medium at 37 °C. After elimination of fat and outer connective tissue, the lymphoid tissue was cut into fine pieces. The minced material was gently teased through a steel sieve into Eagle's MEM (Difco), buffered at pH 7.2 with sodium bicarbonate, and supplemented with non-essential amino acids without glutamine, colimycin 50 U ml⁻¹, and fungizone 10 µg ml⁻¹. Normal calf serum was added at 5% concentration. Aggregates were allowed to settle and the supernatant material was centrifuged at 800 r.p.m. for 10 min. The cells were then suspended in the above culture medium at a concentration varying from 8×10^6 to 2×10^7 ml⁻¹, and distributed in 1,000 ml blood transfusion flasks, each receiving 250 ml of cell suspension. The culture flasks were incubated at 37 °C in a humid atmosphere of CO₂-O₂ (5:95) without agitation.

Viability of the cells, estimated using trypan blue exclusion, in two different cultures is shown in Fig. 1. About 60% of cells died during the first 2–4 months of culture; after this initial period, the viability did not seem to be greatly altered for more than 1 yr. During these long term cultures, only moderate changes in size distribution of the cells were observed. Thus in three 'blind' examinations, we found a slight but reproducible variation in the relative percentages of cells of 5–9 µm and 10–15 µm in diameter. The latter represented 10% of the total viable cell population at day 1 of the culture, but only 5% at day 735. Thus the vast majority of the calf lymph node cells consisted of small and medium sized lymphocytes.

We studied the ability of the lymphoid cell suspensions to respond either to a non-specific mitogen, phytohaemagglutinin (PHA) (Difco), or to specific antigens, sheep red blood cells (SRBC) and φ17 phage, active on *Streptomyces chrysomallus*, strain 17 (ref. 2). With PHA, a moderate stimulation of DNA synthesis, measured by incorporation of ³H-thymidine (2 µCi ml⁻¹ of the culture medium containing 10^6 cells) was observed only during the first days of culture. At the optimal time of incubation (63 h), and with the optimal dose (0.01% final concentration), the stimulation index, calculated as the ratio, (c.p.m. in stimulated sample)/(c.p.m. in control sample), did not exceed 2.8. It should be noted, however, that even in these young cultures the level of incorporation in the control cultures in the absence of PHA (500–600 c.p.m. per 10^6 cells) was lower than that usually found in comparable cultures of mouse or rabbit lymph node cells.

From day 50 to day 58 of culture, the response to PHA was measured after 48 h of contact with the mitogen. As the incorporation in the control cultures without PHA was still lower than that found previously (less than 100 c.p.m. per 10^6 viable cells), no enhancement of incorporation was observed after treatment with PHA. The situation was the same at day 778.

The capacity of the cultures for *in vitro* response to antigens was studied using two methods: the formation of direct plaque forming cells (PFC) after stimulation with sheep red blood cells (SRBC), and the determination of the frequency of response units to φ17 phage³. Stimulation with SRBC was performed using the above culture medium, containing 5×10^{-6} M 2-mercaptoethanol, and two RBCs per calf lymphoid cell.

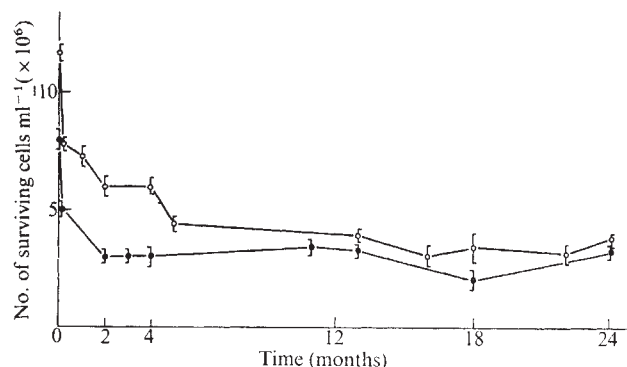


Fig. 1 Survival of calf lymph node cells in two different long term cultures as determined by trypan blue exclusion. Means $\pm$ s.e. from five independent samplings. Bars indicate calculated s.e.

The number of direct PFC was measured each day after stimulation, using the CMC method⁴. No background of PFC was found in the absence of stimulation. In culture to which antigen was added, the maximum level of response was reached at day 9 after stimulation. Four independent determinations of the number of PFC were performed at each period of the culture.

Using young cultures of calf lymph node cells (up to 2–3 weeks) the maximal number of PFC reached was 357 ± 71 per 10^6 cells. It was 342 ± 45 per 10^6 cells after 7 months. Thus, although the number of viable cells had decreased by more than 50%, the proportion of cells able to form plaques remained unchanged during this period. By contrast, after 2 yr of culture, the number of PFC was only 154 ± 47 (a young culture (1–3 weeks) was simultaneously studied and gave from 350 to 450 PFC per 10^6 cells); the situation throughout this period was reversed, compared with the previous one. A 30% decrease in viability took place from day 21 to day 778, whereas a 50% decrease in number of PFC was observed for the same period; thus the proportion of PFC to total viable cells decreased by about 40%.

This effect of ageing on the immunocompetence was confirmed by studying the frequency of response units towards $\phi 17$ phage. The methods used for preparing and statistically analysing microcultures containing variable numbers of active lymphoid cells were as described previously³. Briefly the main characteristic of the method is to preserve good survival of normal lymphoid cells by dilution into irreversibly inactivated cells of the same origin. Inactivation without noticeable alteration of the viability was obtained by previous treatment with streptovitamin (U9361). This procedure permits the use of very small numbers of active lymphoid cells in optimal conditions to carry out a primary *in vitro* response. These cultures are phage-stimulated and are capable of neutralising the phage by producing IgM antibodies. The smaller the number of active cells in the microculture the greater the percentage of negative microcultures. Positive microcultures are considered to contain at least one complete response unit, the frequency of which is calculated. Four independent series of microcultures were carried out in each case. Each series involved sixteen wells for each experimental point. The results were as follows: up to day 245, no noticeable variation in the frequency of response units (1 per 2,100–2,500 cells) was observed. From day 723 to day 770 of culture, the frequency seemed to be considerably altered. In four different experiments we found a value of 1 response unit for 50,000–70,000 cells. Such a reduction during a period of 16–17 months corresponds to the disappearance of more than 95% of cells able to produce antibodies neutralising significantly (more than 20%) $\phi 17$ phage.

The various results reported here must be compared with the results obtained for *in vivo* experiments. Price and Makinodan⁵ have shown that an 80% decrease in the number of direct PFC was observed during the primary response of 30–32-month-old

mice, compared with 3-month-old mice. Such a decrease was assigned mainly to the reduced rate, or capacity, of division of both the B and T cells. Our *in vitro* experiments do not permit us to rule out this hypothesis, although the evolution of the *in vitro* immunocompetence does not seem to be directly related to that of the potentiality of proliferation. Indeed, very few dividing cells seem to be present in the calf-lymph-node cell suspensions, even in the first days after removal from the animal. After PHA stimulation, the level of DNA synthesis is low; however, the immunocompetence, estimated in terms of PFC or anti-phage response units, is high in the young cultures, and does not seem to vary for several months. It is only after the first year of culture that the immunocompetence is affected.

The evolution of immunocompetence thus seems to be independent of that of the proliferative capacity. The specificity of the immune response emphasises the particular significance of these results and supports the hypothesis that the process of ageing expresses intrinsic cellular deficiencies. Moreover, the fact that the frequency of response units towards the phage is more altered than the response measured in number of PFC, suggests that the cells producing large amounts of antibody are relatively less affected by these intrinsic deficiencies. Clearly, these experiments do not disprove the genetic mutation hypothesis of ageing⁶ but rather lend support to some sort of cytoplasmic hypothesis, either Orgel's error catastrophe hypothesis⁷, or the accumulation of cytoplasmic breakdown products⁸ disturbing the mechanisms of antigen recognition and/or antibody biosynthesis.

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MARIE-AGNES DELPIERRE
JACQUES PANIJEL*

Service de Physiopathologie de l'Immunité,
Institut Pasteur, 28 Rue du Dr Roux,
Paris 15, France

Received November 11, 1974.

*To whom requests for reprints should be sent.

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Effect of a protein-free diet on lymph node and spleen cell response *in vivo* to blastogenic stimulants

A PROTEIN-deficient diet determines an early involution of the thymus, and the quantitative difference between lymphocytes from blood, lymph node and spleen of intact and of thymectomised rats is generally smaller with a protein deficient diet than with a normal one^{1,2}. On the other hand, the decrease in the size of blood lymphocytes¹, and in the viability³ of lymphocytes from lymph node and spleen, following deprivation of dietary protein is conditioned to a large extent by the atrophy of the thymus.

These data already militate in favour of a predominant depletion and functional inactivation of the thymus-dependent (T) lymphocytes in experimental protein malnutrition. This conclusion is corroborated by the findings we report here, which show that a protein-free diet inhibits to a much greater extent the lymphocytic activation induced *in vivo* by blastogenic stimulants known to be almost exclusively (phytohaemagglutinin⁴) or partly (sheep red blood cells^{5,6}) thymus-dependent than the

Table 1 Changes in body weights and weights of a popliteal lymph node and of the spleen in normally fed and protein-deprived rats on day 4 after subplantar injection of PHA, LPS and SRBC

Group	Body weight (g)	Normal diet Weight (mg)		Body weight (g)	Protein-free diet Weight (mg)	
		Popliteal lymph node	Spleen		Popliteal lymph node	Spleen
Non-injected controls	306.9 ± 3.8 (7)	7.8 ± 0.6	623.5 ± 31.7	113.6 ± 1.3 (18)	3.4 ± 0.4	138.6 ± 6.5
Injected with PHA	319.3 ± 8.0 (6)	19.9 ± 3.9 2.55	714.2 ± 28.5 1.14	112.3 ± 1.2 (12)	4.0 ± 0.5 1.19	136.6 ± 9.1 0.98
Ratio: injected/ non-injected						
Injected with LPS	322.0 ± 8.4 (6)	12.6 ± 1.3 1.62	709.0 ± 21.8 1.14	117.3 ± 1.5 (10)	3.6 ± 0.8 1.08	209.8 ± 14.4 1.51
Ratio: injected/ non-injected						
Injected with SRBC	301.8 ± 16.7 (6)	20.1 ± 2.3 2.58	663.5 ± 20.2 1.06	112.6 ± 1.6 (12)	6.0 ± 0.8 1.74	113.3 ± 8.3 0.82
Ratio: injected/ non-injected						

Values are means ± s.e. The numbers of rats are indicated in parentheses. The weight changes in the organs due to the stimulants are represented by the ratios between injected and non-injected rats on the same diet.

Composition of the salt mixture in protein free diet: CaCO₃, 18.90; CaHPO₄, 2 H₂O, 12.60; KCl, 14.90; MgSO₄, 8.77; NaH₂PO₄, H₂O, 20.10; NaCl, 22.20; NaI, 0.0026; Na₂SiO₃, 9H₂O, 0.61; FeSO₄(NH₄)₂SO₄, 6H₂O, 1.22; CuSO₄, 5H₂O, 0.44; MnSO₄, 4H₂O, 0.087; ZnCl₂, 0.17 (figures represent percentages).

activation resulting from thymus-independent factors (lipopolysaccharides⁷).

Adult male rats of a pathogen-free Sherman strain were subjected for 2 months either to a balanced normal diet (commercial chow, CNRS, Orléans) or to a protein-free diet. The latter contained (per 100 g dry weight) 73.4 g sucrose, 10 g white dextrine, 10 g peanut oil, 5.7 g salt mixture (ref. 8), 0.5 g choline chloride, 0.06 g inositol, and the following vitamins (in milligrams): thiamine, 1.0; riboflavin, 2.0; niacin, 20.0; Ca pantothenate, 3.0; pyridoxin, 2.0; folic acid, 0.1; vitamin B₁₂, 0.006; biotin, 0.020; tocopherol, 1.0; menadione, 1.0; vitamin A, 5,000 IU; vitamin D, 1,000 IU.

Phytohaemagglutinin, (Eurobio, Paris; PHA), lipopolysaccharides from *E. coli* 055:B5 (Difco, Detroit; LPS) and sheep red blood cells (SRBC) were injected into both hind foot pads of normally fed rats (3 mg of PHA, 12.5 µg of LPS, and 6 × 10⁸ SRBC injected into each foot pad) at doses double those in protein-deprived rats (respectively, 1.5 mg, 6 µg and 3 × 10⁸). The animals were killed on day 4 after injection of PHA, and on days 4 and 8 after that of LPS and SRBC. All rats received intraperitoneally 1 h before death 1 µCi g⁻¹ ³H-thymidine (CEA, Saclay; specific activity 5Ci mM⁻¹).

The mean weights of a popliteal lymph node and of the spleen were determined and the incorporation of ³H-thymidine into these organs was measured in a liquid scintillation counter (Intertechnique, Paris) in the presence of scintisol. The data

obtained in treated rats were compared with those of non-treated ones subjected to the same regimen. On day 8, the responses were very weak even with a normal diet. Therefore only the findings of day 4 were analysed.

The weight of the popliteal lymph nodes and, even more, the incorporation of ³H-thymidine increased strongly on day 4 after injection of each of the three stimulants in normally fed rats. (Tables 1 and 2). The increase was, however, much more marked with PHA and SRBC than with LPS. The activation of the DNA synthesis was obvious even if it was not calculated per lymph node as a whole but per mg of lymphoid tissue. In protein-deprived non-treated rats, the spleen showed a striking drop both in weight and in DNA synthesis, whereas in popliteal lymph nodes, the decreases in weight (Table 1) contrasted with an increased incorporation of ³H-thymidine per mg of tissue (*P* < 0.05) (Table 2). This may correspond to a selective disappearance of non-dividing, probably thymus-dependent, lymphocytes as suggested from previous data^{1,9}.

The lymphocytic activation induced by PHA per entire lymph node was 6.54 times weaker in protein deprived-rats than in controls (× 4.33 against × 28.31), 4.28 times weaker (× 1.24 against × 5.31) after injection of LPS, and 3.81 times weaker (× 4.29 against × 16.38) after SRBC (Table 2). Whereas the spleen was not significantly influenced by PHA, and even reduced and inactivated after injection of SRBC, on a protein-free diet, protein-deprived rats injected with LPS displayed a

Table 2 Incorporation of ³H-thymidine (c.p.m.) by lymphocytes of popliteal lymph nodes on day 4 after subplantar injection of PHA, LPS and SRBC in normally fed rats and in protein-deprived rats

Experimental group	Popliteal lymph node				Spleen			
	c.p.m. per entire organ Normal diet*	c.p.m. per entire organ Protein-free diet†	c.p.m. per mg Normal diet*	c.p.m. per mg Protein-free diet†	c.p.m. per entire organ Normal diet*	c.p.m. per entire organ Protein-free diet†	c.p.m. per mg Normal diet*	c.p.m. per mg Protein-free diet†
Non-injected controls	1890 ± 424	2,427 ± 676	211 ± 56	658 ± 150	780,100 ± 133,900	12,317 ± 1,480	1,288 ± 233	88 ± 8
Injected with PHA	53,508 ± 20,163	10,519 ± 2,313	2,283 ± 523	2,683 ± 544	1,647,100 ± 313,600	13,907 ± 4,233	2,302 ± 430	98 ± 24
Ratio: injected/ non-injected	28.31	4.33	10.83	4.08	2.11	1.13	1.79	1.11
Injected with LPS	10,042 ± 1,714	3,019 ± 852	786 ± 104	814 ± 124	1,097,200 ± 351,390	70,000 ± 30,535	1,527 ± 480	299 ± 122
Ratio: injected/ non-injected	5.31	1.24	3.73	1.24	1.41	5.68	1.18	3.40
Injected with SRBC	30,960 ± 3,590	10,415 ± 2,958	1,641 ± 267	1,563 ± 365	1,083,900 ± 166,700	4,739 ± 2,282	1,608 ± 198	48 ± 37
Ratio: injected/ non-injected	16.38	4.29	7.79	2.37	1.39	0.38	1.25	0.54

*Values obtained from counts made on pools of two lymph nodes.

†Values obtained from counts made on pools of four lymph nodes and two spleens (two rats).

noticeable splenomegaly (1.51 times the weight of the spleen of non-injected controls; Table 1) and a very strong activation of the synthesis of splenic DNA (5.68 times the normals; Table 2). Therefore, the stimulation of the total number of lymphocytes from lymph node and spleen provoked by LPS in protein-deprived rats was not at all weaker than that observed in normally fed rats, contrary to what had been observed with the two thymus-dependent stimulants.

The finding that in protein-deprived rats the lymphocytic response to LPS spread up to the spleen whereas the response to PHA remained almost completely limited to the popliteal lymph nodes, may be attributed to the higher diffusibility of the LPS molecules. In the experimental rats the diffusion was probably facilitated by the atrophy of the lymph node follicles⁹, which normally tend to retain the antigens¹⁰, and thus play the role of a barrier against the dissemination of the latter. The extent of the diffusion of LPS with a protein-free diet was emphasised by the appearance of necrotic areas in the liver of LPS-injected rats. Normally fed rats treated with LPS and protein-deprived ones, non-injected or injected with PHA or SRBC, did not exhibit similar lesions. In addition, doses of LPS double those used in the experiments described above, provoked a 44% mortality with a protein-free diet whereas corresponding doses were inoffensive to normally fed rats.

The inhibitory effect of protein deprivation on the lymphocytic response to SRBC was less marked than the effect on the response to PHA. Indeed, the action of PHA concerned almost exclusively the T lymphocytes whereas SRBC stimulated both the T and B cells, and the inactivation of the latter by the protein-free diet was much less pronounced than that of T lymphocytes².

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A. ASCHKENASY

Centre National de la Recherche Scientifique,
Centre Marcel Delépine,
Laboratoire d'Hématologie nutritionnelle,
45045 Orléans Cedex, France

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Increased hyaluronic acid production on stimulation of DNA synthesis in chick embryo fibroblasts

STUDIES of differentiating tissues indicate that the characteristic products of differentiated cells begin to appear only after cell multiplication has slowed or stopped¹. Therefore, it was concluded that cell multiplication and differentiated function are mutually exclusive. Although this conclusion was based mainly on studies of progressively developing tissues, it has been extended to cells which have already differentiated but have retained the capacity to multiply². This is supported by some older studies of differentiated cells in culture³⁻⁵. There is growing evidence, however, from studies both *in vitro*^{6,7} and *in vivo*⁸, that cell multiplication and differentiated function are compatible in differentiated cells capable of multiplication. Here we add to that evidence by showing that the production of hyaluronic acid, a characteristic product of connective tissue, is positively correlated with the multiplication rate of chick embryo fibroblasts.

Chick embryo cells were cultured as described by Rein and

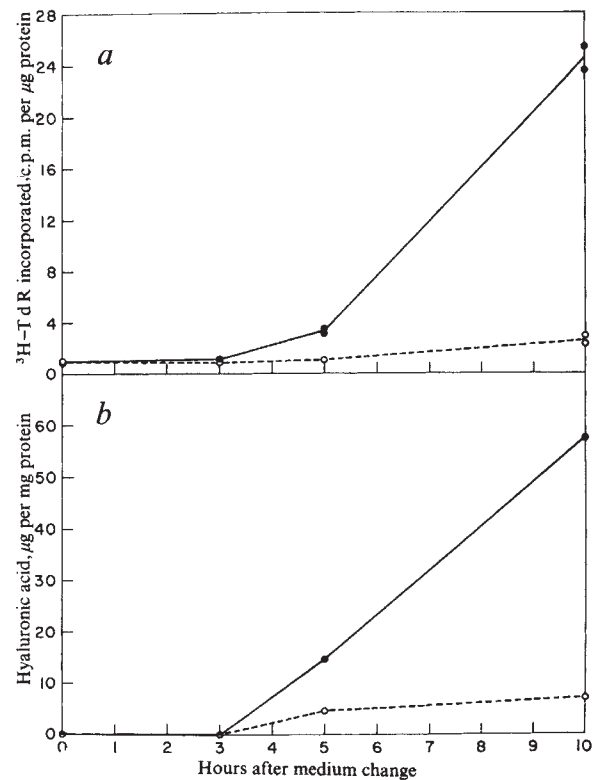


Fig. 1 Four-day-old cultures in 100-mm tissue culture dishes were turned-off by changing to Medium 199, pH 6.8, with 2% (v/v) tryptose phosphate broth and without serum. The pH was controlled by varying the amount of NaHCO₃ in the medium. 16 h later the cultures were washed three times with Earle's salt solution at pH 6.8. Either turn-on medium (Medium 199, pH 7.4, with no tryptose phosphate broth and with 1% (v/v) chicken serum) or turn-off medium (Medium 199, pH 6.8, with no tryptose phosphate broth and with no serum) was added back to the cells. *a*, The rate of DNA synthesis by turned-on and turned-off cultures was measured at various times after the medium was changed by the incorporation of ³H-TdR into acid insoluble material in a 1 h pulse. DNA synthesis was measured under identical conditions by changing all cultures to Medium 199, pH 7.4, with no tryptose phosphate broth and no serum and with ³H-TdR (0.2 µCi ml⁻¹) for the pulse. *b*, Medium was collected from subsets of the cultures at various times after the medium was changed and assayed for the amount of hyaluronic acid accumulated. ●, Turned-on cultures; ○, turned-off cultures.

Rubin¹¹. They were grown at pH 7.4 in Medium 199 with 2% tryptose phosphate broth and 1% chicken serum. The multiplication rates of confluent cultures were controlled by manipulating pH and serum concentration of the medium^{9,10}. Low multiplication rates (turned-off cultures) were obtained by incubating cells for 16 h in serum-free medium at pH 6.8. Cultures were then stimulated to multiply faster (turned-on) by changing to medium (pH 7.4) containing serum and were compared with cultures to which fresh turn-off medium had been restored.

Medium was collected from subsets of the cultures at various times after the change of medium. The cells were then washed once with buffered physiological saline and the washings were added to the collected medium. This mixture was lyophilised and redissolved in a small volume of distilled water. NaOH was added, to a final concentration of 0.1 N, and the solution was incubated overnight at room temperature. Acid mucopolysaccharides were isolated by the procedure of Bollet^{13,14} as modified by Morris¹⁵, giving 90% recovery of a known amount of hyaluronic acid added to Medium 199. Acid mucopolysaccharides were assayed by the method of Dische¹⁶ as modified by Bitter and Ewins¹⁷.

The relative rates of DNA synthesis were measured by the incorporation of ³H-thymidine (³H-TdR) into acid insoluble

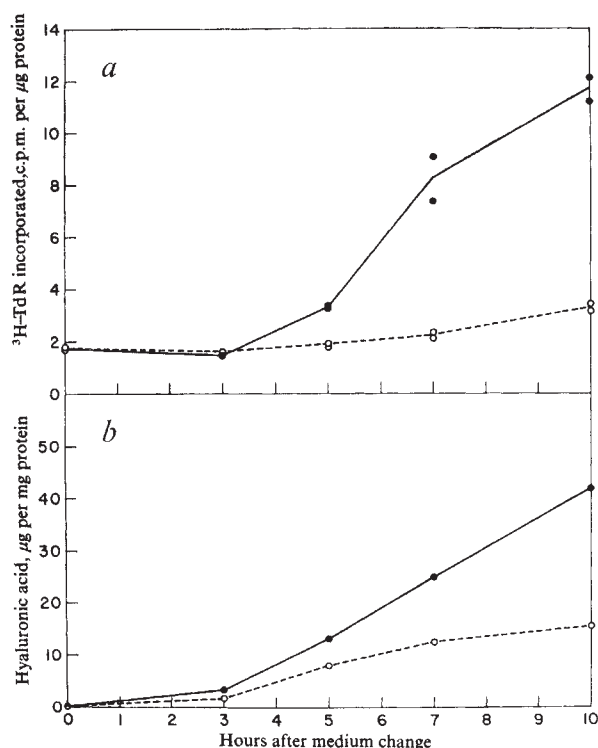


Fig. 2 Four-day-old cultures in 100-mm tissue culture dishes were turned-off by changing to Medium 199, pH 7.4, with 2% (v/v) tryptose phosphate broth and without serum. 16 h later the cultures were washed three times with Earle's salt solution at pH 7.4. Either turn-on medium (Medium 199, pH 7.4, with no tryptose phosphate broth and with 2% (v/v) chicken serum) or turn-off medium (Medium 199, pH 7.4, with no tryptose phosphate broth and with no serum) was added back to the cells. *a*, The rate of ^3H -TdR incorporation at various times after the medium was changed, measured as in Fig. 1. *b*, The amount of hyaluronic acid accumulated in the cultures at various times after the medium was changed. ●, Turned-on cultures; ○, turned-off cultures.

material in a 1-h pulse¹¹. The overall rate of DNA synthesis of a culture reflects the proportion of cells in the S phase of the cell cycle at any given time, and, therefore, is proportional to the rate of multiplication of the culture¹².

The cultures kept in medium at pH 6.8 with no serum continued to synthesise DNA slowly during the 10 h of measurement (Fig. 1*a*). Cultures given medium at pH 7.4 with 1% serum had doubled their rate of DNA synthesis at 5 h, and had increased it almost tenfold at 10 h. These turned-on cultures had also accumulated three times as much hyaluronic acid as the turned-off cultures at 5 h, and eight times as much at 10 h (Fig. 1*b*). The insensitivity of the assay at hyaluronic acid levels less than 2 μg mg⁻¹ cell protein prevented accurate measurements of hyaluronic acid production earlier than 3 h after the medium change.

To show that the differences in hyaluronic acid production were not merely an effect of pH, cells were turned-off by incubating them for 16 h in serum-free medium at pH 7.4. They were then turned-on by adding serum-containing medium at the same pH. When serum concentration alone was varied, there was less difference in DNA synthesis rates between turned-on and turned-off cultures than when both pH and serum concentration were changed (Fig. 2*a*). The turned-on cultures were synthesising DNA at a distinctly faster rate than the turned-off cultures at 5 h, and reached a 3.5-fold greater rate at 10 h. The amount of hyaluronic acid accumulated was detectably greater in the turned-on cultures at 5 h and had become almost three times as great at 10 h (Fig. 2*b*).

These results show that the rate of cell multiplication and expression of differentiated function, as represented by hyaluronic acid production, are positively correlated with one

another. We have found this situation to continue for at least 24 h after stimulation, by which time almost all the cells have undergone division. Although we have not yet shown that DNA synthesis and hyaluronic acid production are positively correlated in a single cell, we conclude that there is no basis for postulating an incompatibility between multiplication and differentiated function in this system.

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DAVID MOSCATELLI

HARRY RUBIN

Department of Molecular Biology and Virus Laboratory,
University of California, Berkeley,
California 94720

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Mitotic delay in the slime mould *Physarum polycephalum* induced by low intensity 60 and 75 Hz electromagnetic fields

INTEREST in the biological effects of non-ionising radiation has increased in the past few years, with most workers focusing on radio frequency and microwave regions of the spectrum¹⁻³. The ubiquity of low level 50-60 Hz electromagnetic radiation in the environment is a strong reason to investigate the biological effects of extremely low frequency radiation^{4,5}. We have examined the effects of low intensity 60 and 75 Hz electromagnetic fields (EMFs) and have observed delays in the mitotic cycle of the myxomycete *Physarum polycephalum* after continuous exposure. Significant delays were observed after approximately 100 d of continuous exposure, corresponding to about 300 mitotic cycles.

We used *Physarum polycephalum* because (1) its normal life cycle has been documented extensively both morphologically and biochemically, (2) the desired vegetative or reproductive stages can be induced at the discretion of the investigator and (3) stationary cultures undergo naturally synchronous mitosis. In synchronous mitosis an entire culture of approximately 10⁹ nuclei simultaneously undergoes mitosis, permitting accurate observation of cycle timing^{6,7}. Synchronous mitosis occurs at predictable intervals so that the time between mitoses can be used as a sensitive indicator of EMF effects. Stationary macroplasmidia can be obtained by allowing a sample of microplasmidia from submerged shake-flask cultures to coalesce on filter paper supported by milk-filter disks; growth medium is added after coalescence. Both submerged shake-flask cultures and stationary macroplasmidia were exposed continuously to EMFs.

Radiation fields were simulated by application of crossed electric and magnetic fields alternating in phase at 60 or 75 Hz, at levels of 0.7 V m⁻¹ and 2.0 gauss, selected to correspond to similar fields which may be generated by the US Navy's proposed Project Sanguine communications antenna⁸. Our field intensities are a factor of ten greater than ground-level intensities

projected in 1970 for the Sanguine antenna; ambient 50–60 Hz fields arising from both natural and artificial sources are often within an order of magnitude of these intensities. EMFs near electrical appliances in the home are often⁹ in the range 10–25 gauss and 2–200 V m⁻¹.

The magnetic field was generated by means of four large, thin rectangular coils placed parallel to each other at regular intervals; the entire working region was exposed to a uniform alternating field. Electric fields were produced by applying a small voltage (~40 mV) to parallel stainless steel electrodes in contact with the growth medium. Each container in the incubator (shake flasks and macroplasmia dishes) was equipped with its own, individually adjustable pair of electrodes. Direct electric currents were blocked by a capacitor; alternating currents through the cell were about 0.2 mA. As a check on possible electrolysis of the growth medium, the pH of several flasks containing only growth medium was monitored for several days and found to be unchanged. Control cultures and cultures exposed to EMFs were maintained in separate incubators at temperatures of 25.5±0.3 °C. The incubators were identical in every respect except that field coils and plates were not activated in the control incubator. Stock lines of *P. polycephalum* were maintained as microplasmia in submerged shake-flask cultures in both the simulation and control incubators; cultures were removed from the incubators only to transfer them to fresh growth medium or to withdraw samples for stationary macroplasmia. As soon

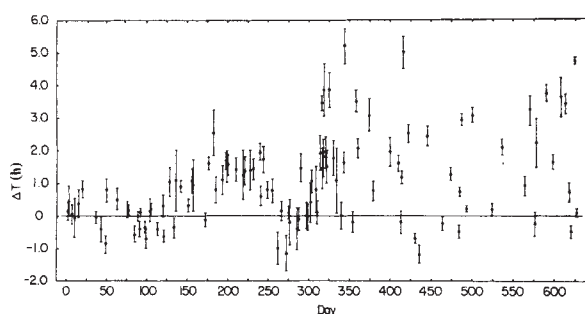


Fig. 1 Relative time required for stationary cultures of *P. polycephalum* to reach the metaphase configuration of mitosis compared with the number of days of continuous exposure to 75 Hz EMFs (0.7 V m⁻¹, 2.0 gauss). Each point represents the average of 10 cultures from which the average of 10 control cultures for that day has been subtracted. The error bars are 95% confidence limits.

as stationary cultures were made, both submerged and stationary cultures were returned to simulation and control incubators. To eliminate small day-to-day variations in mitotic cycle timing an identical number of stationary control cultures was set up each time observations were to be made on a group of experimental cultures. All cultures were handled in an identical fashion using aseptic techniques. The experimental parameter was the time required for stationary cultures to reach metaphase of the second mitotic division measured from the addition of growth medium. The second division was used because it occurs approximately 14 h after feeding, so that observations can be scheduled in the morning. Periodic measurements of the interval between the second and third mitoses confirmed cycle delays ascertained from observations of the second mitosis alone.

After a culture had been in a 60 Hz EMF for 80–100 d a significant mitotic delay became evident, while the same effect took 100–120 d in a 75 Hz EMF. To determine whether the change in the mitotic cycle was reversible, several cultures were moved from the simulator to the control incubator. The delay persisted for 30–60 d during which time the mitotic interval slowly converged to the control interval. No significant delay was observed in cultures exposed for 200 d to weaker 75 Hz EMFs of 0.15 V m⁻¹ and 0.4 gauss. Furthermore, when cultures with a delayed cycle were put in these fields after being taken

from the higher intensity 75 Hz simulator, the delay slowly decreased and became insignificant after 40 d.

Figure 1 presents data for cultures exposed to 75 Hz EMFs; data for 60 Hz fields are similar. The dip in the curve during days 260 to 310 results from difficulties with control cultures, which became excessively slimy and underwent mitosis asynchronously; on day 310 they were replaced with alternative cultures that had been maintained in the control incubator from day 1 as a reserve.

The data were analysed by two statistical tests; both test the null hypothesis that experimental and control populations are identical. An analysis of variance was used to apply a test of significance simultaneously to differences between all possible pairs of day-averages. In other words, the question posed is: can a horizontal line be drawn through the data (not necessarily at $\Delta = 0$)? A signed ranks test was also used; in the test, the error bars on the data points are ignored and the number and extent of positive differences are compared with negative differences to determine if any net effect is present. On some days mitosis took less time to occur in experimental than in control cultures. This test asks whether the reversal happened often enough to accept the null hypothesis. Our analysis shows that for both 60 and 75 Hz data, both statistical tests indicate that the null hypothesis can be rejected with at least 95% confidence. The 75 Hz data for days 260–310 were included in our analyses.

We conclude that low level, low frequency EMFs can influence biological systems. This conclusion agrees with findings from other laboratories^{4,5}. Two important features of our observations deserve emphasis. First, delay in the mitotic cycle does not show up until after the cultures have been exposed for long periods. Second, the delay does not disappear immediately when the cultures are removed from the field simulator.

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MICHAEL T. MARRON
E. M. GOODMAN
BEN GREENBAUM

Division of Science,
University of Wisconsin-Parkside,
Kenosha, Wisconsin 53140

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Rapid quantitation of membrane antigens

STUDIES of cell-surface antigens are important in several fields of biological research, notably transplantation and tumour research. The most commonly used tests for such antigens involve assays requiring complements, and the subsequent measurement of cellular damage by the release of radioactive isotopes or the uptake of supravital dyes. Radioimmunoassays which measure directly the antibody-antigen reaction in such studies would have considerable advantages. Although it is possible to label antibody molecules directly, such attempts have so far been unsuccessful because of high background binding values. Furthermore, variations in the composition of the antisera would make each antiserum a unique target for radiolabelling with consequent variations in results. We have

Table 1 Use of ^{125}I -protein A for rat, mouse and human cell typing

Rat target	Sera	c.p.m.	Mouse target	Sera	c.p.m.	Human target*	Sera†	c.p.m.
Lewis (Le)	DA→Le Le→DA	7,400 340	H-2 b	k→b	4,676	2, 5, 12, 19	1	282
						2, 5, 12, 19	7	183
						2, 5, 12, 19	2	1,538
DA	DA→Le Le→DA	541 6,920	H-2 k	k→b	604	2, 5, 12, 19	12	1,045
						2, 5, 12, 19	28	761
DA×Le (Fi)	DA→Le Le→DA	3,810 3,520	k×b (Fi)	k→b	2 514	3, 7, 28, W15	1	265
						3, 7, 28, W15	7	1,073
						3, 7, 28, W15	2	791
						3, 7, 28, W15	12	299
BD	DA→Le Le→DA NRS	542 3,376 387	P815 H-2 d NMS	k→d	7,771 470	3, 7, 28, W15	28	1,140
						1, 3, 7, W15	1	934
						1, 3, 7, W15	7	881
						1, 3, 7, W15	2	300
						1, 3, 7, W15	12	317
						1, 3, 7, W15	28	114

Cells in tubes or microtitre test plates were incubated with (25 μl) antisera for 30 min at 4 °C, washed and incubated with ^{125}I -labelled (50,000 c.p.m.) protein A or ^{125}I -labelled whole bacteria. After a further 30 min at 4 °C the cells were washed and counted. Cells on plates were washed by centrifugation followed by shaking out the supernatant, and transferred to tubes for counting. Rat, mouse and human sera were used at dilutions of 1:125, 1:100 and 1:5, respectively. The P815 mouse mastocytoma cells were from *in vitro* cultures.

*HL-A type was determined by Dr Bertil Lindblom at the University Hospital using "Uppsala" antisera and a trypan blue cytotoxicity test.

†Anti-HL-A sera from McIndoe Memorial Research Unit, East Grinstead, Sussex, UK.

Arrows represent anti.

tried to circumvent this using a radiolabelled homogeneous marker for IgG molecules produced by certain bacteria.

Protein A from *Staphylococcus aureus* Cowan I binds specifically and with high affinity to the Fc portion of most mammalian IgG subclasses¹. In our assay ^{125}I -labelled *Staphylococcus aureus* Cowan I or labelled protein A were incubated with cells precoated with a wide range of antisera to membrane components. Radioactivity (c.p.m.) provides a measure of the number of IgG molecules bound per cell and hence the number of specific probe-surface markers. The value of protein A as an immunological probe lies in its versatility, as in our example, in which a single ^{125}I -labelled protein A preparation has been used to study antigens on human, rat and mouse cells precoated with rabbit and monkey as well as with alloantisera.

The preparation and iodination of protein A have been described elsewhere². Whole bacteria were isolated in a similar manner but were simply washed free of excess iodine. We have used this ^{125}I -protein A to develop a complement-free radioimmunoassay applicable to a wide range of mammalian membrane antigens. Table 1 shows how the method can be used to type human, mouse and rat lymphoid cells with alloantisera or xenoantisera. The data for rat and mouse show the expected gene dose effect as observed under optimal reaction conditions using both antibody and ^{125}I -protein A in excess. Such a gene dose 'control' provides a good test of the function of any individual experiment. Cross reaction of Lewis anti-DA with BD rats is also clearly shown.

Thus, for rat and mouse systems, using inbred material, the

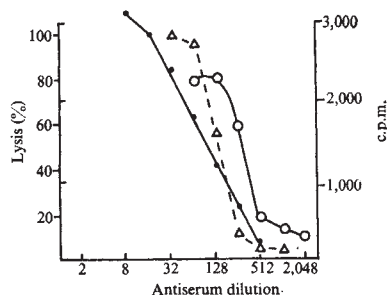


Fig. 1 Triplicate dilutions of rabbit anti β -2 microglobulin were assayed for ability to bind to 10^5 human T cells by three separate methods. The ^{51}Cr assay⁴ (○) and trypan blue test (△) are plotted as percentage lysis because these assays measure cell death. The protein A assay (●) measures directly the number of antibody molecules bound per cell, and the cells remain viable throughout; c.p.m. bound at each antiserum dilution are therefore plotted. Sensitivity of the IpA assay can be varied simply by changing the specific activity of the iodinated reagent.

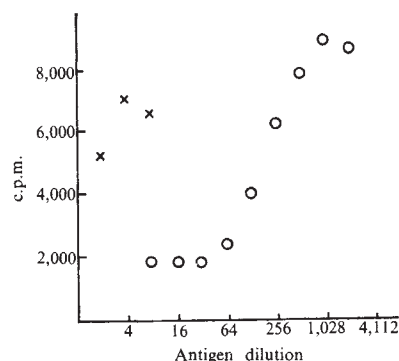


Fig. 2 HL-A antigen isolated by standard procedures⁵ from splenic lymphocytes after HN_4Cl removal of red cells, was diluted with saline and a constant amount of antibody added (sufficient to give 10,000 c.p.m. per 10^6 cells) with ^{125}I -protein A. After 2 h at 4 °C, 10^5 cells were added per well of the test plate and incubation continued for a further 30 min at 4 °C. Plates were centrifuged, washed and incubated with protein A in the usual way. ○, HL-A 2 antigen; ×, HL-A 12 antigen. Antiserum used, A macacus anti HL-A 2 serum⁶.

results of this assay were highly satisfactory. Table 1 shows that the assay can also function in the HL-A system, although sizeable binding occurs between supposed 'negative' serum-cell combinations. Extensive cross reactions can occur, however, between HL-A antigens; this is in agreement with the cross reaction found³ between HL-A2 and HL-A28. When we compare (Fig. 1) the protein A assay with conventional assays for HL-A such as trypan blue or ^{51}Cr using microassays in plates⁴, we found a positive correlation between the three tests. The ^{125}I -protein A assay has a similar sensitivity to that of the trypan blue or ^{51}Cr assays, but is more linear and thus more precise than the other two tests.

The ^{125}I protein A assay was also tested for its suitability in testing for soluble HL-A determinants in inhibition assays. Figure 2 indicates that the protein A assay is very suitable for studies of HL-A antigens in soluble form. The inhibitor assay obtained was again more linear than inhibition of trypan blue or ^{51}Cr lysis.

Whole *Staphylococcus aureus* bacteria bind to the Fc region of IgG and there seemed to be no reason why bacteria could not be heavily labelled, for example, for cell suicide experiments. Also cells carrying whole bacteria can be readily separated by Ficoll gradient centrifugation (V. Ghetie, K. Nilsson, and J. Sjöquist, unpublished), from those not carrying bacteria. In preliminary studies we have iodinated bacteria and used them in an identical manner to that used for protein A. Figure 3

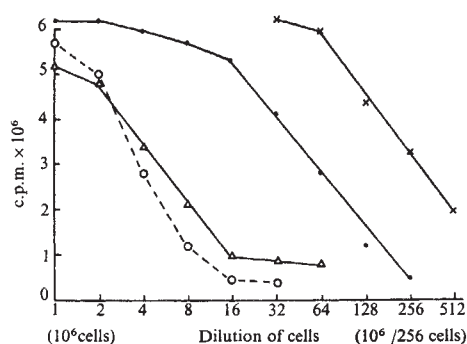


Fig. 3 Dilutions of T and B cells in microtitre test plates were assayed directly for binding of ^{125}I -labelled whole bacteria or preincubated with rabbit anti-Ig sera and assayed for binding of ^{125}I -labelled whole bacteria. The assay conditions are as described in the footnote to Table 1. $\circ$, T cells + autologous serum; Δ , T cells incubated with anti-Ig; $\bullet$, B cells + autologous serum; $\times$, B cells incubated with anti-Ig.

shows such an assay involving human B and T cells. B cells or T cells separated by T cell rosettes⁶ were incubated with normal human sera or rabbit anti-Ig sera before testing.

Using ^{125}I -labelled *Staphylococcus aureus* Cowan I strain bacteria, human B lymphocytes incubated with anti-Ig antibodies bind approximately four times the number of bacteria than do uncoated B lymphocytes, and some 100 times more than T lymphocytes treated or not with anti-Ig. Note that our antisera contained antibodies against light chains (both χ and λ chains) but still failed to react with human T lymphocytes as assessed by this assay (in other words, there is no difference between T lymphocytes treated or not with anti-Ig).

We have described mainly how ^{125}I -protein A assays can be used to supplement conventional methods in mammalian systems for assaying cell-bound and soluble antigens. Several points make protein A assays unique, perhaps the most important being the specificity of binding to Ig molecules. Protein A will only bind to IgG 1, 2 and 4 in human cells, there being no binding to IgG 3 or IgM for example. In general IgG 1, 2 and 4 account for about 92% of human IgG. We have carried out a series of experiments in which human cells precoated with alloantibodies are then coated with rabbit anti-IgG before addition of protein A. Such sandwich techniques, however, hardly increase specific binding in the human system. In the rat and mouse system it is not known exactly what percentage of complexed IgG binds protein A but our sandwich assays indicate it to be 60–100% in both species. Calculation of the absolute number of antigen molecules per cell are, of course, affected by such considerations but this is easily checked by including a sandwich assay as described above. The assays described in Table 1 and Figs 1 and 2 approach linearity and we expect total linearity when the assays improve technically. This makes them much more amenable to kinetic studies and also to studies of cross reaction than complement-dependent assays. In addition cells are live at the end of the experiment and loss of counts from cells can be easily monitored.

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K. I. WELSH

McIndoe Research Unit, Queen Victoria Hospital,
East Grinstead, Sussex, UK

G. DORVAL

Department of Tumour Biology, Karolinska Institute,
104 01 Stockholm 60

H. WIGZELL

Department of Immunology, Uppsala University,
Box 562, 751 22 Uppsala 1, Sweden

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Membrane changes in a circadian system

Njus *et al.*¹ have recently suggested a membrane model of a biological clock and propose that the clock mechanism is a feedback oscillator in which the ion transport channels envisaged in the fluid mosaic model of cellular membranes² are involved directly. It is postulated that the proteins which make up these channels respond to changes in concentration gradients of specific ions in the membrane, by grouping to form transport channels when the gradient is small, and dispersing to non-transporting modes when the gradient is large. Thus the protein distribution in the membrane could be regulated by the oscillating ionic gradient and could itself regulate the ionic gradient to complete the feedback pathway and drive the oscillation. It has also been suggested³⁻⁵ that oscillatory changes at cellular membranes may be directly associated with the clock mechanism, based on observations that the phase or the period of the oscillation can be changed by treatments which affect the passage of ions through membranes.

We provide direct experimental evidence for cyclic changes in membrane properties in a circadian system from studies of the pulvinule cells at the base of the leaflets of clover, *Trifolium repens* L. Swelling of the cells in the adaxial portion of the pulvinule and compression of those in the abaxial portion cause the leaflet to open, and a reversal of this process causes it to close. We have shown⁶ that the volume changes and the resulting leaf movements are caused by an endogenous circadian oscillator located in the pulvinule. Further observations (which will be reported more fully elsewhere), have shown that redistribution of cations (principally K^+) within the pulvinule accompanies these variations in volume.

Table 1 Ion concentrations in pulvinal tissue (meq per kg wet weight)

	Adaxial	Abaxial	Adaxial—Abaxial
K	Day <i>193.7 ± 11.7</i>	167.8 ± 8.3	25.9 ± 9.2
	Night 140.3 ± 12.5	<i>197.4 ± 31.2</i>	−57.1 ± 20.8
Na	Day 19.5 ± 2.7	18.5 ± 2.5	0.9 ± 2.2
	Night 19.5 ± 2.4	<i>24.6 ± 5.7</i>	−5.2 ± 4.1

Mean ion concentrations ($\pm$ s.e.m.) of abaxial and adaxial segments of pulvinal tissue of clover in LD 12:12. The swollen (convex) segment is shown in *italics*.

Table 1 gives the ion concentrations in pulvinal tissue for plants grown in a 12 h light, 12 h dark cycle (LD 12:12) at 20 °C. The pulvinules were sectioned into abaxial and adaxial segments at about the middle of the light and the dark intervals, and analysed using flame photometry. The cation component is predominantly potassium and examination of sections stained with sodium cobaltinitrite show that most of it is in the cortical pulvinal cells which occupy at least 80% of the volume of the segments. As the potassium concentration is significantly greater on the swollen side where there are fewer cells per unit volume, it is clear that each enlarged cell contains very much more potassium than each cell on the contracted side. Thus there must be large tidal movements of potassium across the pulvinal membranes during the daily cycle. Using a different experimental technique, Satter, Galston and co-workers conclude that similar potassium changes occur in *Albizia*⁷ and *Samanea*⁸.

Radioisotope tracer studies reveal that considerable changes in potassium transport occur in pulvinal tissue during the cycle. Leaves were soaked in a medium containing ^{42}K and ^{45}Ca for 2 h and washed for 30 min; their pulvinules were sectioned

into abaxial and adaxial segments. The activity of the segments from groups of several pulvinales was measured immediately, and again after the short lived ^{42}K had decayed, to determine separately the influxes of the two ions. The double labelling experiment was performed because it was found that calcium influx is approximately proportional to the dry mass of the tissue segments, which is more appropriate for comparison purposes than the wet mass which fluctuates as the cells expand and contract. Since the dry mass is difficult to measure accurately for a small number of tissue segments, the potassium influx could more conveniently be expressed as its ratio to the calcium influx.

Figure 1 shows the variation in influx ratio for the two sides of the pulvinule during an LD 12:12 cycle. The influx of potassium is relatively much greater when the cells are expanded than when they are contracted. Such differences are expected for cells in the process of swelling or shrinking. The influx ratio is, however, significantly greater or less than unity for much longer periods which commence before the leaf is observed to move and continue for several hours after the leaf is fully opened or fully closed. It has not been practicable to obtain corresponding efflux data.

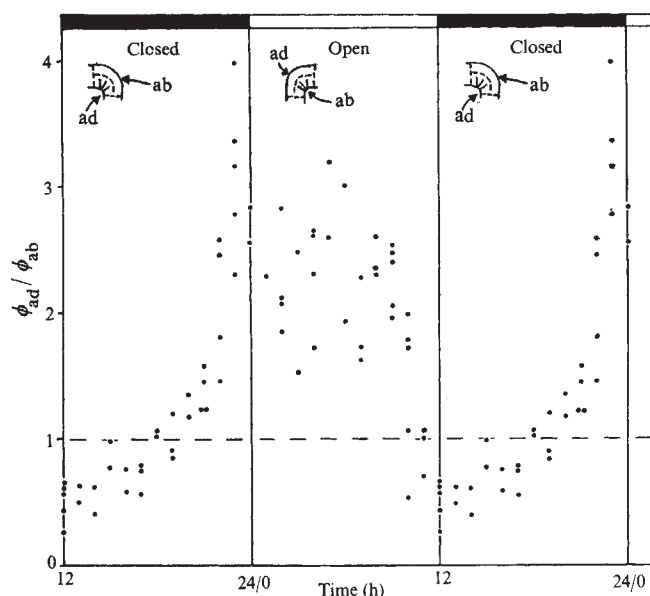


Fig. 1 Variation of pulvinal influx ratio with time (h after onset of light) for clover grown in LD 12:12 at 20 °C. ϕ_{ad} is the K^+ influx per unit influx of Ca^{2+} in adaxial segments of pulvinales. ϕ_{ab} is the corresponding quantity for abaxial segments. Each point is the influx ratio for groups five to ten pulvinales. One and a half cycles of the oscillation are shown.

These data show that the transport properties of the membrane are changing in a cyclic manner in LD 12:12. The changes may be in membrane permeability to specific ions or in an active transport system. The phase of the influx cycle is about 3 h in advance of both the leaf oscillation and the LD cycle. This phase relationship is significant since it strongly suggests that the change in membrane properties is a preliminary step in the sequence of changes responsible for leaf movement, presumably by osmotically varying the volume of pulvinal cells through changes in their potassium content.

The phase relationship in LD 12:12 also suggests that the flux changes are not a direct response to the light cycle. This is confirmed by transferring plants to conditions of constant light and temperature (LL), following which we found that the influx oscillation continues for at least two cycles. The period of these oscillations is about 27 h, which is the same as the period of leaf oscillations under these conditions⁶. We therefore conclude that the oscillatory system controlling both potassium influx and leaf movement is circadian and under endogenous control.

Attempts have been made to measure the transmembrane

potentials of pulvinal cells. The internal potential of cells on the abaxial side is -71.6 ± 2.0 mV early in the contracted phase and -102.1 ± 6.4 mV early in the swollen phase (P. S. Targett, unpublished). Cells on the adaxial side show corresponding changes but opposite in phase. The time course of these potential changes has not yet been established in detail; but it corresponds qualitatively to what is expected if the membrane potential is determined principally by the passive diffusion of potassium, since the magnitude of the potential is larger when the internal potassium concentration, and presumably the concentration gradient, is higher.

In their circadian oscillator model, Njus *et al.*¹ do not suggest a mechanism for the dispersal of the proteins forming the transport channels when the concentration gradients are large. We propose that the electric field in the membrane may act as the dispersing agent by supplying the necessary forces. The average strength of the transverse field is large ($\approx 10^7$ V m^{-1}), but the field lines are likely to be distorted in the vicinity of transport channels where hydrophilic pathways will have much lower resistance per unit area than the bulk of the membrane. The distorted field will have components in the plane of the membrane. The proteins immersed in the membrane, which make up the transport channels, carry charged endings and therefore would experience forces resulting from a lateral field which could cause them to disperse or undergo changes in configuration when the electric field increases.

It is well known that depolarisation of the excitable membranes in nerve⁹ and the large Characean cells¹⁰ causes large changes in the passive fluxes of specific ions, resulting in an action potential. Hyperpolarised membranes also show marked changes in their conductance to specific ions¹¹. Eskin¹² has suggested that the biological clock in *Aplysia* may involve neuronal depolarisation.

Experimental support for a circadian oscillator located at the cellular membrane in some biological organisms does not preclude the existence of a different system sited elsewhere (for example, in the nucleus¹³) which controls other oscillatory characteristics in these or other organisms. If rhythmicity gives a biological system an evolutionary advantage, it is quite possible that different mechanisms have arisen independently.

B. I. H. SCOTT
H. F. GULLINE

Department of Physics,
University of Tasmania,
Hobart, Tasmania, Australia

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Effects of L-dopa metabolites at a dopamine receptor suggest a basis for 'on-off' effect in Parkinson's disease

ALTHOUGH L-dopa is the most effective therapeutic agent available for the control of the akinesia of Parkinson's disease, long term therapy may be associated with a variety of therapeutic problems¹. One problem seen in some patients is a progressive loss of therapeutic response which may be associated with abnormal involuntary movements². A state may develop in which a good response to the drug alternates with periods of akinesia and rigidity^{3–4}. This has been called the

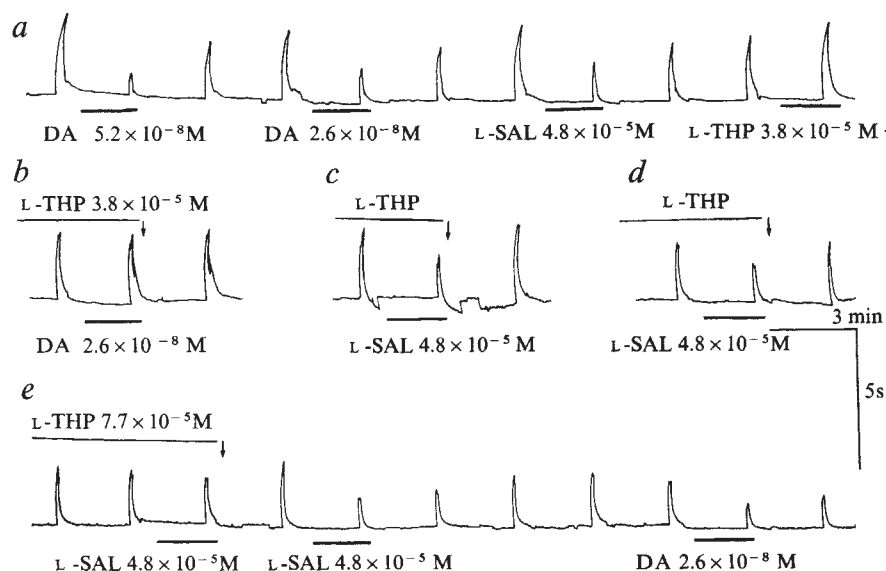


Fig. 1 Effects of dopamine (DA hydrochloride, Mann) L-salsolinol (L-SAL) and L-THP on intestinal responses evoked by electrical stimulation of gut of *Tapes watlingi*. Guts were prepared as before⁹, incubated in artificial seawater and stimulated electrically by means of transmural electrodes. Unlabelled upward deflections represent contractions of the gut resulting from a 5-s train of square wave pulses (1 ms, 20 Hz, 10 V). The black bars indicate the periods for which DA, L-SAL or L-THP were present in the 2-ml organ bath. a, Perfusion with artificial seawater alone. b, c and d, Responses 4 min after L-THP (3.85×10^{-5} M) had been added to the bath. e, L-THP (7.7×10^{-5} M) was also present. After the times marked by arrows L-THP was washed from the bath.

'on-off' effect or akinesia paradoxa¹. While low blood levels of L-dopa may explain the onset of akinesia ('off' effect), this cannot explain the sudden reversal of patients' akinesia ('on' effect) which may occur without additional dosage of L-dopa^{2,3}.

Several mechanisms have been proposed to explain both the progressive loss of efficacy and the 'on-off' effect, but so far there is little supporting data¹⁻⁴. One suggestion has been that complex metabolites of L-dopa, which are structurally similar to apomorphine, may antagonise the effects of dopamine⁵. Two complex metabolites of L-dopa, tetrahydropapaveroline (THP) and salsolinol (SAL) have been identified in the urine of patients with Parkinson's disease who had been treated with L-dopa⁶. THP has also been identified in rat brain after administration of L-dopa⁷. It has been suggested, however, that THP, SAL or similar tetrahydroisoquinoline compounds may behave as dopamine (DA) agonists and contribute to the therapeutic effect of L-dopa^{6,8}.

We have now found that THP may antagonise the action of DA and SAL at a DA receptor. We have investigated the activity of DA and the isomers of THP (hydrochloride salt), SAL (hydrobromide salt)¹³ and amphetamine at DA receptors in the isolated gut of the mollusc *Tapes watlingi*, which contains both dopaminergic neurones and specific dopamine receptors⁹. Figure 1 shows the responses to electrical stimulation in one gut preparation in the presence of DA, L-SAL and L-THP. Contraction was inhibited by DA (2.63×10^{-8} – 5.26×10^{-8} M) and L-SAL (4.8×10^{-5} M), but was hardly affected by L-THP (3.85×10^{-5} – 7.7×10^{-5} M) (Fig. 1a). The DA-like effect of L-SAL suggested that this compound was a weak agonist at the DA receptor. In two out of six preparations in which L-THP had no DA-like effect, the amplitude of contraction increased. It is interesting that DL-THP has been reported to have no DA-like effect on adenylyl cyclase from rat striatum¹⁰.

In the presence of L-THP (3.85×10^{-5} M), the inhibitory response to DA (2.63×10^{-8} M) was abolished (Fig. 1b), while the response to L-SAL (4.8×10^{-5} M) was unaffected (Fig. 1c and d). A larger concentration of L-THP (7.7×10^{-5} M) did reduce the response to L-SAL (Fig. 1e). In two further preparations, L-THP (2.31×10^{-5} M and 7.7×10^{-5} M) completely antagonised the inhibitory effects of L-SAL (2.4×10^{-5} M and 9.6×10^{-5} M, respectively). These concentrations of L-THP also antagonised DA at an equipotent concentration (5.26×10^{-8} M). Clearly, the effects of both DA and L-SAL can be antagonised by L-THP.

D-THP was relatively inactive as a DA antagonist in concentrations ten times greater than those at which L-THP was effective. It was equally effective when the concentration of D-isomer was 30 times greater than that of the L-isomer. Both D and L-THP have antagonist activity at DA sensitive receptors

associated with adenylyl cyclase of the caudate nucleus¹¹. When tested on five gut preparations, DA was 10^3 times more potent than equimolar concentrations of L-SAL. In two experiments, the optical isomers of SAL were approximately equipotent as DA agonists. The optical isomers of amphetamine (amphetamine sulphate, Sigma) had a DA-like effect on the preparation. Again, the isomers were equipotent and exhibited molar potencies similar to those measured for isomers of SAL. Recent studies of the DA-sensitive adenylyl cyclase system in the rat striatum revealed either no DA-like activity of SAL¹⁰ or DA antagonism by L-SAL¹¹. The reasons for the failure to demonstrate DA agonist activity with D- and L-SAL in DA-sensitive adenylyl cyclase of the rat striatum is not clear. One possible explanation is the limited range of concentrations tested. Alternatively, if SAL acts as an indirect agonist, the lack of dopaminergic nerve terminals in the preparation would exclude such an effect.

Histochemical fluorescence⁹ has indicated that the dopaminergic system in the gut of *Tapes watlingi* consists of varicose nerves with a presynaptic capacity for the synthesis, release and uptake of DA. There are, in addition, postsynaptic receptor sites which respond to DA agonists. As SAL lowers the accumulation and retention of tritiated DA in rat brain synaptosomes¹², it is possible that the optical isomers of SAL mimic the effect of DA on the gut of *Tapes* by augmenting its release and/or inhibiting its reuptake.

Thus we have demonstrated the diverse pharmacological activity of known metabolites of L-dopa. D- and L-SAL are dopamine agonists and L-THP is a potent dopamine antagonist.

We suggest that the therapeutic effects of L-dopa that are presumed to be mediated by DA may be modulated by metabolites of DA itself. Thus therapeutic success or failure with L-dopa in Parkinson's disease may depend in part on the relative rates of production and accumulation of these or similar metabolites in the brain.

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DON DOUGAN
DENIS WADE
PETER MEARRICK

University of New South Wales,
Department of Clinical Pharmacology,
St Vincent's Hospital,
Sydney, New South Wales, Australia

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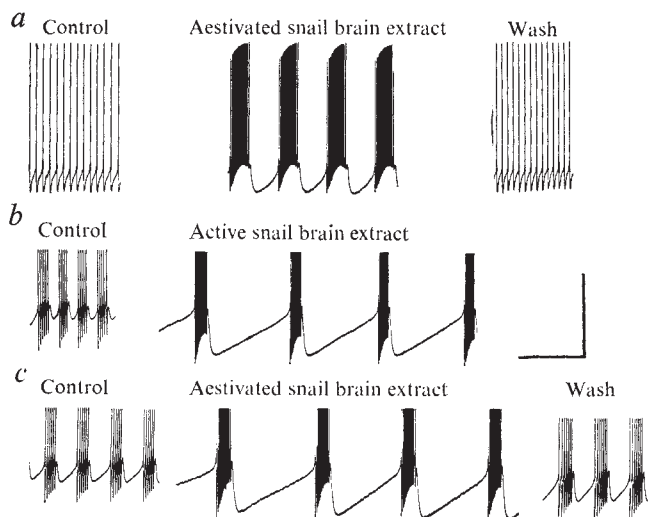
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Peptide factor extracted from molluscan ganglia that modulates bursting pacemaker activity

NEUROHORMONES have been suggested as long term regulators of the physiology and biochemistry of nervous tissue¹⁻³. Long term changes in the electrophysiology and biochemistry of a specific neurosecretory cell in the land snail *Otala lactea*^{4,5} suggested such a form of long term hormonal regulation, and in fact several vertebrate neurohypophysial peptides cause long term changes in the membrane properties of this cell⁶. The regulatory effects observed involve the induction or potentiation of bursting pacemaker potential (BPP) activity, resulting in net excitation of the cell. These effects are different from the conventional transient conductance changes produced by various putative transmitters on these cells. We have now found a naturally occurring substance present in the snail brain that produces similar long term changes in the membrane properties of this neurosecretory cell. The results provide evidence for a peptide neurohormone which may function physiologically in the seasonal regulation of the physiology and biochemistry of a neurosecretory cell.

Crude extracts of molluscan nervous tissue were prepared by removing the circumoesophageal ganglia from *Otala lactea* and *Aplysia californica*, and rinsing the tissue in physiological saline. For comparison, the buccal retractor muscle and foot muscle of the snail were also excised. The tissue was blotted on filter paper, weighed, and transferred to a 5% acetic acid solution in a glass homogenising tube, immersed in a bath of boiling water.

Fig. 1 Effects of snail brain extracts on BPP activity of cell 11. *a*, Membrane potential traces of cell 11 (taken from a dormant snail) exposed to an extract of whole brain from aestivated snails. Under control conditions the snail could not generate BPP activity. A 5-min bath application of the extract induced BPP activity. Washing for more than 1 h with extract-free saline restored the activity to approximately its control levels. *b* and *c*, Extracts of brains from either aestivated or active snails augmented the BPP activity already present in cell 11 taken from an active snail. Prolonged washing restored the membrane potential behaviour to its control state. Calibration: 40 mV, 12 s.



The tissue was heated for 10 min in this solution, and then homogenised twice using a Teflon-tipped pestle. The ratio of extraction solvent to tissue was 20:1 (v/v, tissue specific gravity assumed to be 1.0). The homogenate was centrifuged at 12,000g for 15 min, and the supernatant was removed and lyophilised. The dried material was resuspended in either snail physiological saline⁴ (80mM NaCl, 4mM KCl, 10mM CaCl₂, 5mM MgCl₂, 10mM Tris, pH 7.8) for assay, or in 5% acetic acid for separation on Sephadex G-10 (0.9 × 43 cm) or G-25 (0.9 × 30 cm) columns. The resulting fractions were lyophilised and dissolved in snail saline for assay. Sephadex columns were standardised with blue Dextran 2000, yellow Dextran 20 (Pharmacia), cytochrome c, vitamin B₁₂, cobalt chloride, lysine-vasopressin, oxytocin and various amino acids as markers. Elution volumes of the peptides and amino acids were determined by spotting aliquots of the various fractions on Whatman 3 MM filter paper, and spraying with ninhydrin.

Assays were performed on the peptide-sensitive neurosecretory cell (cell 11)⁶, taken from either active or dormant snails. Dissection of the ganglion, identification of cell 11, and recording techniques were as described previously⁴⁻⁶. Cells taken from dormant snails have different membrane properties from those taken from active snails⁵, and therefore the samples to be tested were applied to both types of preparations. The basal level of spontaneous electrical activity in the cell was usually recorded for 30-60 min before application of the material to be tested. The 1-ml samples (and control samples) were added by pipette to the 4.0 ml saline bathing the snail ganglion. After observation of the effect, the preparation was washed with 20 volumes of physiological saline.

After application of the extract to the medium surrounding a cell from a dormant snail generating a beating pacemaker rhythm, BPP activity was induced (Fig. 1a) relatively rapidly (seconds to minutes). Application of the extract to a cell from an active snail potentiated BPP activity already present (Fig. 1b and c). The response of the cell was initially increased during the washing. Prolonged washing of the cell (> 1 h) in saline was usually required to reverse the effects of the extract. Thus, the factor(s) in the extract had a long term effect on the BPP activity of cell 11 similar to that observed with neurohypophysial peptides⁶. The increase in BPP activity observed immediately on washing may have resulted from the removal of inhibitory substances (for example, the putative transmitters dopamine and glutamate, both of which inhibit BPP activity in cell 11) which acted only while the extract was in the bath. Extracts with activity were obtained from ganglia of active (Fig. 1b) and dormant (Fig. 1c) snails and from the circumoesophageal ganglia of *Aplysia californica* (not illustrated). Similar extracts of the buccal retractor and foot muscles of the snail had no effect, suggesting a brain-specific substance.

Preliminary attempts were made to characterise this factor(s). The BPP activating factor was lost during dialysis, but maintained full activity when exposed to 100 °C for 10 min or to 50% acetic acid. These treatments had similar effects on vasopressin's ability to induce or enhance BPP activity. Fractionation of the crude extract on Sephadex G-10 showed that the active factor(s) was eluted in the exclusion volume (V_E) (Fig. 2a) whereas a potent inhibitory factor was eluted in subsequent elution volume (V_1). Since Sephadex G-10 excluded molecules with molecular weights greater than 700, we conclude that the BPP inducing factor is greater than 700 while most of the inhibitory material is smaller. In standard runs on Sephadex G-10, lysine-vasopressin and amino acids eluted in V_E and V_1 , respectively. Fractionation of the crude extracts on Sephadex G-25 (which excludes molecules greater than 5,000) yielded an inactive exclusion volume (V_E) (Fig. 2b). BPP-inducing activity was in the V_3 fraction (Fig. 2b). (This fraction also contained the vasopressin and oxytocin in standard column runs.) In addition, there seemed to be a larger inhibitory factor in V_2 as well as inhibitory material in V_3 (since washing the cell free of V_3 further enhanced BPP). The latter inhibition most likely corresponds to the components found in V_1 from Sephadex G-10

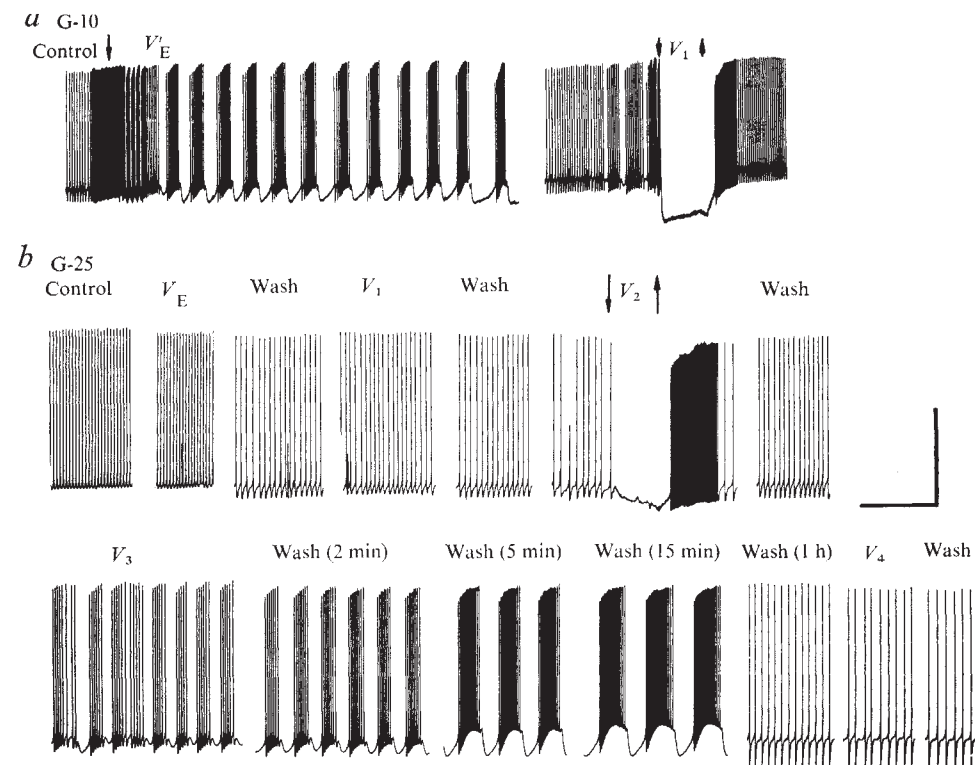
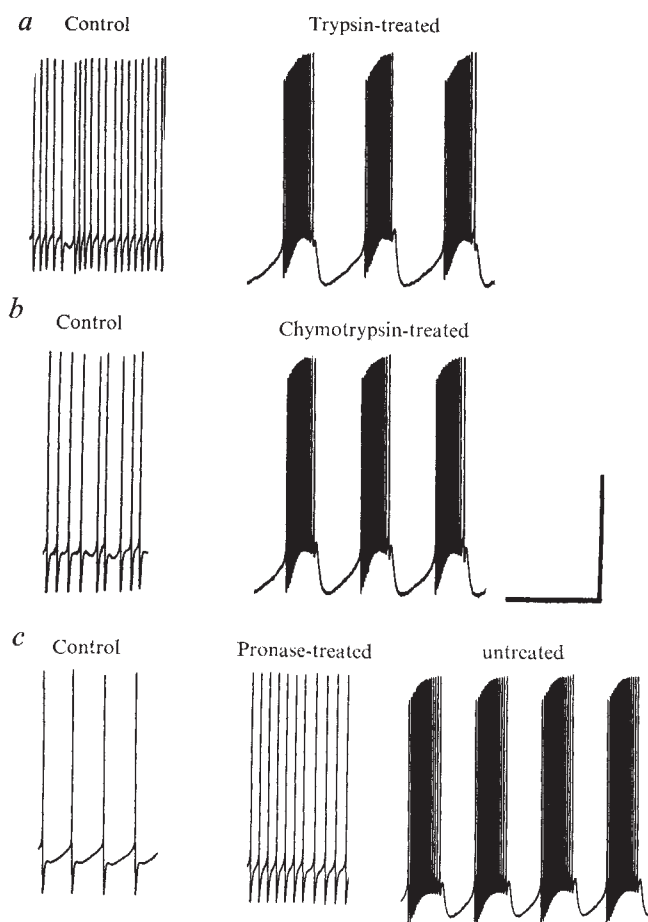


Fig. 2 Sephadex chromatographic separation of snail brain extracts. *a*, Application (arrow) of fraction V_E (exclusion volume) from Sephadex G-10 induced BPP activity (with large amplitude slow oscillations, which generated high frequency spike activity), whereas application of the V_1 (included volume) caused inhibition of spontaneous activity and hyperpolarisation of cell 11, which was reversible washing with fresh saline (upward arrow). *b*, Application of various fractions from a Sephadex G-25 separation showed that the active factor(s) was in the V_3 fraction. Another, higher molecular weight factor with a slight inhibitory effect on the BPP was eluted in V_2 . Note that washing the cell after application of V_3 produced an enhancement of the BPP (this may have been caused by the presence of amino acids which eluted in both V_3 and V_4 —see text for further details). Calibration: 40 mV, 12 s (2 min during part of control, and V_1 wash in (*a*) and V_2 wash in (*b*)).

Fig. 3 Effects of proteases on the BPP-inducing factor. *a*, Electrical activity in cell 11 before (control) and after application of trypsin-treated factor. *b*, Same as (*a*), except the material was treated with chymotrypsin. *c*, Electrical activity in cell 11 before (control), after application of the Pronase-treated extract, and after application of an equivalent amount of the extract not treated with Pronase (untreated). Calibration: 40 mV, 12 s.



(Fig. 2a), since in the standard runs amino acids eluted in the V_3 fraction. The final fraction (V_4) was inactive.

The pharmacological effects and gel filtration behaviour of the activating factor in the extract suggest that the extracted factor might be a peptide. We therefore investigated whether the extracted factor could be inactivated by proteases. Aliquots of the active V_E fraction from Sephadex G-10 (Fig. 2a) were incubated with immobilised trypsin or chymotrypsin (Worthington) or Pronase (Calbiochem). Preliminary experiments showed that all these proteases inactivated the BPP-inducing activity of vasopressin (Pronase and chymotrypsin inactivated oxytocin, but trypsin did not), and that enzyme treatment itself did not affect the assay. Incubation of the active extracts with either chymotrypsin or trypsin did not inactivate the BPP-inducing factor (Fig. 3a and b), but Pronase treatment abolished activity (Fig. 3c). These results indicate that the active material was a peptide, but not vasopressin or oxytocin since both of these peptides were inactivated by chymotrypsin (and trypsin, in the case of vasopressin).

In summary, we have isolated from molluscan ganglia a factor(s) which can either induce or enhance BPP activity in a specific neurosecretory cell in *Otala lactea*. The activating effects of this peptide factor are similar to the actions of certain vertebrate neurohypophysial peptides in that both are elaborated by nervous tissue; cause long term changes in the membrane properties of a specific cell in the snail, the result of which is BPP activity; are water-soluble; are stable to heat and acid; are dialysable, and co-elute from Sephadex G-10 and G-25. The extracted factor differs from the neurohypophysial peptides in its resistance to inactivation by trypsin and chymotrypsin. These results suggest that peptides similar, although not identical, to the neurohypophysial peptides function naturally in the modulation of the physiological properties of nerve cells in the snail.

MARK S. IFSHIN
HAROLD GAINER
JEFFERY L. BARKER

Behavioral Biology Branch,
National Institute of Child Health and Human Development,
National Institutes of Health,
Bethesda, Maryland 20014

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Temperature adaptation in myosin of Antarctic fish

A RELATIONSHIP exists between the thermostability of fish skeletal muscle myosin, actomyosin and myofibril preparations and the environmental temperature at which the fish lives¹⁻³. Compared with those isolated from mammals and warm sea fishes, the myosins isolated from cold water species readily aggregate on storage, are more sensitive to denaturation by heat and urea, and quickly lose all ATPase activity following preparation^{4,5}. We have compared the properties of myofibrils and myosin prepared from the white muscle of an Antarctic fish, *Notothenia rossii*, South Georgia, British Antarctica, with homologous preparations from a tropical species, *Amphiprion sebae* (Indian Ocean; 23–27 °C). We suggest that the thermal lability of cold-adapted fish myosins arises from differences in the higher order in the structure of the molecule; this is probably an evolutionary response to attain high catalytic efficiency at low temperatures.

The myofibrillar ATPase enzyme from the Antarctic fish (Fig. 1) has a much higher specific activity at low temperatures (0–10 °C) than does the tropical fish. The apparent energies of activation for the reactions between 0 and 18 °C were

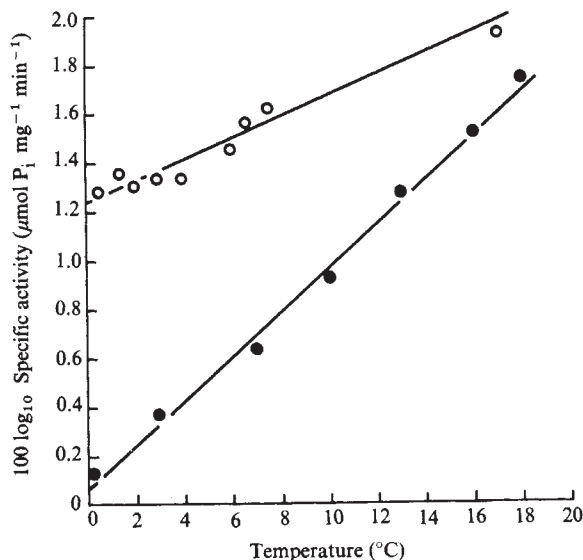


Fig. 1 The effect of temperature on the specific activity of white muscle myofibrils of *Notothenia* (○) and *Amphiprion* (●). Myofibril preparations were used for ATPase activity studies because the ATPase of myosin isolated from cold water fish is extremely unstable. Myofibrils were prepared from fish expial white muscle⁶, excluding superficial red muscle which has a different myofibrillar ATPase activity⁷. Studies on the thermal denaturation of myofibrillar ATPase activity were carried out between 25 and 37 °C by incubating myofibrils (1 mg ml⁻¹) in 0.05 M KCl, 40 mM Tris-HCl (pH 7.5). Myofibrils were added to an 18-fold excess of continuously stirred medium and an initial sample taken 0.5 min later. Subsequent samples were taken at appropriate intervals and pipetted into tubes at 0 °C to prevent further inactivation. Myofibrils partially inactivated by exposure to high temperatures were assayed for ATPase activity at 18 °C. The assay for ATPase activity was performed in 1.5 ml 40 mM Tris-HCl (pH 7.5) with 6 mM ATP, 6 mM MgSO₄ and 0.2 mM CaCl₂ at *I* = 0.124 (adjusted with KCl), and at a myofibril concentration of 0.4–0.5 mg ml⁻¹. The reaction was terminated with 1.5 ml TCA and the liberated phosphate was determined⁸. Determinations of myofibrillar ATPase activity were made at temperatures between 0 and 18 °C in the same assay conditions. Appropriate controls and reagent blanks were included in all experiments.

lower in the case of the Antarctic fish, 7.4 kcalorie mol⁻¹ compared with 17.3 kcalorie mol⁻¹. *Notothenia* myofibrils lost 95% of their activity after 7 min incubation at 37 °C, whereas those of *Amphiprion* still retained 45% of their original activity after 20 min incubation. The denaturation reaction (Fig. 2) followed first order reaction kinetics only in the case of the Antarctic fish. In the tropical fish there was an initial activation of the ATPase activity lasting approximately 12 min; the activation in this species occurred between 37–32 °C. A similar activation effect of much shorter duration was noticed in *Notothenia* at somewhat lower temperatures (32–27 °C).

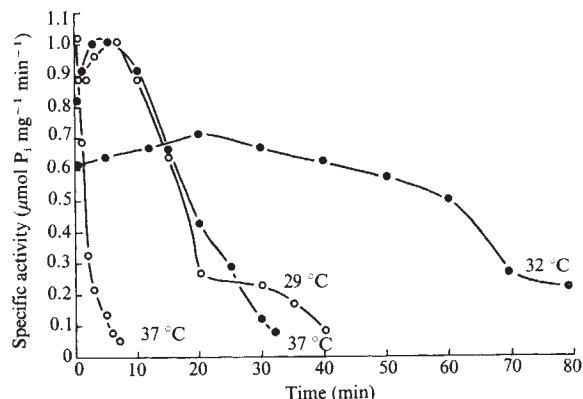
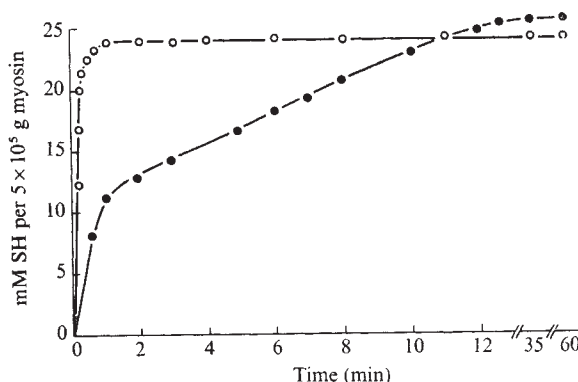


Fig. 2 The thermal inactivation of myofibrillar ATPase of *Notothenia* (○) at 37 °C and 32 °C and of *Amphiprion* (●) at 37 °C and 29 °C.

Myosins were prepared from the white muscle of the two species by a rapid method⁹. The time-dependent exposure of SH groups of myosin (Fig. 3) was determined by measuring the increasing optical density (λ = 412 nm) of the thiophenol anion with 5,5'-dithio bis-2-nitrobenzoic acid (DTNB) following reaction in 0.4 M KCl, 0.1 M phosphate buffer pH 8.0 (refs 10 and 11). The SH groups of *Notothenia* myosin became available for interaction with DTNB very much faster than those of *Amphiprion*, the reaction time being less than 1 min for the Antarctic fish compared with approximately 15 min for the tropical species.

Thus, differences in activation energy (E_a), positively correlated with adaptation temperature, may play an important role in the evolutionary temperature adaptation of the enzyme. Similar differences in E_a are important factors in evolutionary rate compensation in other enzyme systems^{12,13}. It has also been suggested that the higher catalytic efficiencies between homologous enzyme systems from cold adapted poikilotherms compared with homeotherms may be explained in terms of an increase in weak bond formation of the activated enzyme-substrate complex in poikilotherms¹⁴. On this basis the num-

Fig. 3 The time-dependent exposure of myosin SH groups from *Notothenia* (○) and *Amphiprion* (●) measured as the increasing absorbance (412 nm) of the thiophenol anion of DTNB at pH 7.0.



erous weak bonds involved in stabilising the higher orders of structure of cold adapted poikilotherms necessarily make the molecule more susceptible to thermal inactivation at higher temperatures. This inactivation is probably associated with the disruption of certain hydrogen bonds and hydrophobic interactions between intraprotein amino acid residues, resulting in critical changes in the tertiary structure of the protein, causing loss of enzymic activity. We suggest that the higher activity of the myofibrillar ATPase of Antarctic fish at low environmental temperatures is associated with weaker bonding; in other words, with a more open molecular structure. In the case of the tropical fish a more compact and rigid structure seems to be necessary to give the molecule thermal stability at higher environmental temperatures. Evolutionary temperature adaptation of myosins from different fish may well have important implications in limiting the present day geographical distribution of the different species.

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I. A. JOHNSTON

Research Unit for Comparative Animal Respiration,
University of Bristol, Bristol BS8 1UG

N. J. WALESBY

British Antarctic Survey,
Monks Wood Experimental Station,
Abbots Ripton, Huntingdon

W. DAVISON
G. GOLDSPIK

Muscle Research Laboratory, Department of Zoology,
University of Hull, Hull HU6 7RX, UK

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Metabolic and insulin-releasing activities of D-glucose anomers

GRODSKY *et al.*¹ proposed that glucose stimulates insulin secretion because the sugar is metabolised in the pancreatic β cells; this view of stimulus recognition was later termed the substrate-site hypothesis². The hypothesis has received support from observed correlations between rates of insulin release and various parameters of glucose metabolism in the pancreatic islets. It is particularly striking that a triose, D-glyceraldehyde, has been shown to mimic the electrophysiological³ and insulin-releasing^{4–6} actions of glucose; D-glyceraldehyde is oxidised and dilutes ¹⁴CO₂ arising from D-(U-¹⁴C)-glucose in the islets^{4–6}. Although the substrate-site hypothesis has been criticised (for example, refs 7 and 8), many of the arguments raised may be doubtful for reasons discussed elsewhere⁹. An important challenge to the hypothesis is the observation that the α anomer of D-glucose is more effective than the β anomer in stimulating insulin secretion^{10,11}. For the anomeric specificity of the D-glucose recognition system to stand up as evidence against the substrate-site hypothesis, however, it must be shown that the transport, phosphorylation or further metabolism of glucose does not exhibit the same anomeric specificity in the β cell. We report here that β -D-glucose is at least as effective as α -D-glucose in stimulating counter-transport of 3-O-methyl-D-glucose,

Table 1 Effects of D-glucose anomers on the islet content of glucose-6-phosphate

Sugar	μmol glucose-6-phosphate per kg dry weight		
	Primary data	Difference from control	β minus α
None (control)	87.7 ± 18.2	—	—
α -D-glucose 6 mM	150.7 ± 23.3	63.0 ± 8.4†	—
β -D-glucose 6 mM	242.5 ± 41.8	154.8 ± 27.7†	91.8 ± 22.3*
None (control)	81.3 ± 6.3	—	—
α -D-glucose 18 mM	183.5 ± 32.9	102.3 ± 31.7*	—
β -D-glucose 18 mM	213.5 ± 24.6	132.3 ± 30.7*	30.0 ± 47.9

Mean values ± s.e.m. of four different mice are given in each of two experimental series. Values denote glucose-6-phosphate concentration in islets after 3 min incubation without (control) or with D-glucose anomer at the concentrations indicated. In addition to data for each group of islets, the mean ± s.e.m. of differences between parallel incubations are given. Levels of significance by *t* test for paired observations: **P* < 0.05, †*P* < 0.02, ‡*P* < 0.01. Two-way classification (animal/sugar) and analysis of variance of data with 6 mM anomers yielded: *P* < 0.05 for α -D-glucose against control, *P* < 0.001 for β -D-glucose against control, and *P* < 0.01 for β -D-glucose against α -D-glucose.

increasing the islet content of glucose-6-phosphate, and diluting ³H₂O that results from the metabolism of D-(5-³H)-glucose.

Figure 1 shows that α -D-glucose (Sigma) was more effective than β -D-glucose (Sigma) in stimulating insulin release. Both anomers were capable of increasing the islet content of glucose-6-phosphate, but the effect of 6 mM β -D-glucose was significantly greater than that of equimolar α -D-glucose (Table 1). When islets were incubated with D-(5-³H)-glucose, the production of ³H₂O fell on addition of either non-radioactive anomer (Table 2). In keeping with the greater effect of β -D-glucose than of α -D-glucose on glucose-6-phosphate, the effect on ³H₂O production was statistically significant for the β anomer but not for the α anomer. Neither anomer induced any measurable counter-transport of 3-O-methyl-D-glucose at 37 °C (Table 3). This lack of effect probably resulted from an extremely rapid equilibration across the β cell plasma membrane resulting in an almost immediate obliteration of any driving anomer gradient. When the activity of the transport system was reduced by lowering the temperature to 8 °C, both anomers induced a significant counter-transport of 3-O-methyl-D-glucose, but there was no demonstrable difference in effect between the two.

These results indicate that the anomeric preference of the insulin-releasing D-glucose recognition system is not shared by the earliest steps of glucose metabolism. It follows that the discriminatory behaviour of the recognition system probably cannot be reduced to that of the glucose-transporting carrier or the glucose-phosphorylating enzymes, although direct studies of the hexokinase kinetics remain to be performed. This conclusion seems to create a serious difficulty for the substrate-site hypo-

Table 2 Effects of D-glucose anomers on ³H₂O production from D-(5-³H)-glucose

Group	mmol per kg dry weight		
	Primary data	Difference from control	β minus α
Control	9.26 ± 0.88	—	—
α -D-glucose	7.68 ± 0.76	−1.58 ± 1.17	—
β -D-glucose	6.62 ± 0.95	−2.64 ± 0.66*	−1.06 ± 1.08

Mean values ± s.e.m. of five different mice are given. Islets incubated for 10 min with 17 mM D-(5-³H)-glucose in albumin-free medium produced ³H₂O corresponding to 6.09 ± 0.90 mmol glucose (with same specific radioactivity as in medium) per kg dry weight of islets. Table shows production of ³H₂O during same incubation followed by 5 min incubation with 17 mM D-(5-³H)-glucose alone (control) or in combination with 6 mM non-radioactive D-glucose anomer. As results are expressed in terms of glucose with a fixed radioactivity per mol, decreases from control values indicate dilution of the metabolically active glucose pool. In addition to data recorded for each group of islets, the means ± s.e.m. for differences between parallel incubations are given.

*Level of significance by *t* test for paired observations: *P* < 0.02.

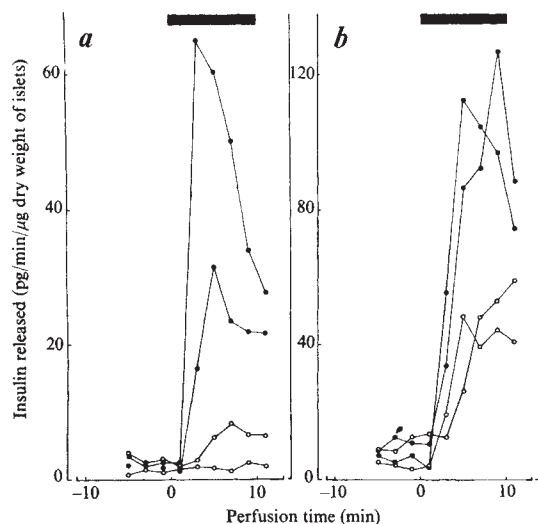


Fig. 1 Insulin release in response to D-glucose anomers. Islets were perfused with glucose-free medium without (a) or with (b) 2 mM theophylline. During periods indicated by bars, the medium also contained α -D-glucose or β -D-glucose. In each experiment the two anomers were tested in consecutive periods separated by 30 min of perfusion with glucose-free medium. In (a) α -D-glucose was tested before β -D-glucose; in (b) the order was reversed. To facilitate comparisons, the periods of perfusion with glucose have been superimposed on the time scales. Each point represents the average rate of insulin release in response to α -D-glucose (●) or β -D-glucose (○) over a period of 2 min. The total concentration of glucose (α plus β) measured in the effluent was 9.0 ± 0.1 mM (8.6–9.2) in studies of the α anomer, and 8.8 ± 0.1 mM (8.3–9.2) in studies of the β anomer. Two separate experiments are shown in each diagram.

Fresh islets were microdissected free-hand from adult non-inbred Umeå-ob/ob-mice; these islets contain more than 90% β cells. In most incubations the basal medium was a Krebs–Ringer bicarbonate buffer kept at 37 °C, equilibrated with ambient air, and supplemented with 20 mM N-hydroxyethylpiperazine-N¹-2-ethane sulphonic acid (HEPES) to stabilise the pH at 7.4. In

thesis, but we do not think that it is sufficient reason for abandoning that hypothesis altogether. A case for caution can be made in the light of some recent experiments on anoxic islets. In such islets the concentration of fructose-1,6-diphosphate does not change with glucose in the concentration range of 1–20 mM. Yet, the glycolytic flux seems to depend markedly on glucose concentration in that range (B. Hellman, L.-Å. Idahl, J. Sehlin, and I.-B. Täljedal, unpublished). Although the rate of islet glycolysis is often assumed to be mainly controlled by glucose phosphorylation, those observations suggest the possibility of a more complex metabolic recognition system for glucose.

Another reason for caution is that rejection of the substrate-site hypothesis would require a change in our explanation of glyceraldehyde-induced and dihydroxyacetone-induced insulin release. The action of D-glyceraldehyde differs from that of D-glucose in not being inhibited by mannoheptulose or glucosamine^{4,5}. As these inhibitors probably act on the islet hexokinases, a metabolic step that is likely to be circumvented by

transport experiments, Krebs–Ringer bicarbonate buffer equilibrated with O₂–CO₂ (95:5) was used. Unless otherwise stated, the media contained 1 mg of bovine serum albumin ml⁻¹. Polarimetric observations of D-glucose anomerisation rates at 37 °C agreed with results of Niki *et al.*¹⁰.

To study insulin release, the perfusion apparatus described previously¹² was modified to enable solutions of glucose anomers to be injected into a stream of basal medium passing groups of 4 islets at a rate of 35 μ l min⁻¹. The glucose solutions were prepared and kept at 0–2 °C for 5 min before injection; the volumes injected were small enough for the temperature to equilibrate with that of the apparatus (37 °C) before glucose reached the islets about 30 s after injection. Insulin was assayed radioimmunologically, and the dry weight of islets was determined as described previously¹².

To measure glucose-6-phosphate, batches of four islets were incubated for 30 min in 25 μ l of basal medium only. Crystals of pure anomers were quickly dissolved in basal medium and warmed for 15 s before being added to the islets; 3 min later the islets were removed and frozen. After freeze-drying (–40 °C, 0.1 Pa) overnight and weighing, islets were extracted and glucose-6-phosphate was determined¹³.

Production of ³H₂O from D-(5-³H)-glucose was measured as an index of the combined glycolytic and pentose phosphate shunt fluxes¹⁴. Batches of three to four islets were incubated for 30–40 min in basal medium and subsequently for 10 min in 15 μ l of medium containing 17 mM equilibrated D-(5-³H)-glucose (2.0 Ci mol⁻¹). An aliquot (5 μ l) of medium containing the labelled glucose alone (control) or with non-radioactive α - or β -D-glucose anomer was then added. After 5 min, metabolism was stopped with 5 μ l 0.27 M HCl. The ³H₂O produced was allowed to equilibrate overnight with 500 μ l water that was in an enclosing outer glass vial. ³H₂O was determined by liquid-scintillation counting, and the islets were dried and weighed as above.

Affinity for the D-glucose transport system was determined as the capacity of D-glucose anomers to induce counter-transport of 3-O-methyl-D-glucose at 37 °C or 8 °C. After incubation in glucose-free medium for 30–40 min, batches of three islets were equilibrated with 1 mM 3-O-methyl-D-(1-³H)-glucose (15 Ci mol⁻¹) and 0.75 mM L-(1-¹⁴C)-glucose (10 Ci mol⁻¹) during incubation in 200 μ l medium for 10 min. After adding 2 μ l of buffer, with or without the non-radioactive glucose anomer, incubation was continued for 1 or 5 min. Islets were removed, freeze-dried overnight and weighed. Their content of ¹⁴C and ³H was determined by liquid-scintillation counting and intracellular 3-O-methyl-D-glucose estimated by correcting for ³H in the L-glucose (extracellular^{15–17}) space.

D-glyceraldehyde, the difference between glucose and glyceraldehyde is readily explained by the substrate-site hypothesis. If one abandons this hypothesis, however, one must postulate some direct sugar receptors that will accept a few, but not many, hexoses and that will also accept trioses. Those receptors must be assumed to bind mannoheptulose in such a way that the receptor's response to glucose is blocked, while that to glyceraldehyde is not. Whether or not one postulates one complicated receptor common to glucose and trioses, or different receptors for glucose and mannoheptulose on the one hand and for trioses on the other, the ensuing hypothetical construct is so complicated as to be unattractive in the absence of solid evidence of a positive kind. So far, results that have been interpreted as possibly reflecting properties of direct glucose receptors in the β cells may be suggestive but are far from conclusive^{18,19}. Only further experiments can tell whether the answer lies in a substrate-site hypothesis developed to conform with the present data, a regulatory-site hypothesis based on direct receptors, or a combination of such mechanisms^{2,4}.

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L.-Å. IDAHL
J. SEHLIN
I.-B. TALJEDAL

Department of Histology,
University of Umeå,
S-901 87 Umeå, Sweden

Received December 2, 1974.

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Table 3 Induction of 3-O-methyl-D-glucose counter-transport by D-glucose anomers

Temperature	No. of experiments	mmol 3-O-methyl-D-glucose per kg dry weight	Control	α -D-glucose	β -D-glucose
37 °C	8		1.35 $\pm$ 0.14	1.40 $\pm$ 0.12	1.49 $\pm$ 0.18
8 °C	7		1.56 $\pm$ 0.21	0.80 $\pm$ 0.10*	0.85 $\pm$ 0.10*

Mean values $\pm$ s.e.m. are given for the numbers of mice indicated. Incubations were performed at 37 °C or 8 °C. Islets were equilibrated with 1 mM 3-O-methyl-D-glucose and subsequently incubated for 1 min (37 °C) or 5 min (8 °C) with or without 6 mM D-glucose anomer. Table shows the intracellular content of 3-O-methyl-D-glucose at the end of incubation.

*Level of significance by *t* test: $P < 0.01$.

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BCG immunotherapy of rat tumours in athymic nude mice

CONSIDERABLE emphasis is now being placed on the use of bacterial adjuvants such as bacillus Calmette Guérin (BCG) and *Corynebacterium parvum* for the immunotherapy of malignant disease. While general immunostimulation by these adjuvants produces a therapeutic response against some but not all experimental animal tumours^{1,2}, there is evidence to suggest that a more effective suppression of tumour growth can be achieved by contacting the adjuvant with the tumour^{3,4}. Tumour cells injected into compatible hosts in admixture with BCG often do not produce progressively growing tumours⁴ and in some cases regression of established tumour grafts can be produced by intralesional injections of BCG (ref. 3) or *C. parvum*⁵. Clinically this is akin to the observation that cutaneous melanoma nodules may regress following intralesional injection of BCG (refs 6 and 7). Adjuvant contact immunotherapy has also been used in experimental animal studies to suppress growth of tumours developing in pulmonary or pleural sites, which are models for the treatment of metastatic disease⁸⁻¹⁰.

Host responses are involved in 'adjuvant-contact'-mediated suppression of tumour growth since the adjuvants are not directly cytotoxic for tumour cells. The relative roles of specific immunity directed against tumour-associated rejection antigens and possibly less specific reactions such as those mediated by activated macrophages is not fully understood. This is emphasised by studies showing that growth of several transplanted rat tumours, including a carcinogen-induced mammary carcinoma AAF57 (ref. 11), could be suppressed by contact with BCG, although these tumours were inactive or only weakly immunogenic as defined by their capacity to induce immunity against

transplanted tumour cells¹². Moreover, BCG-contact suppression of transplanted rat osteosarcomas has been observed in hosts immunosuppressed by adult thymectomy and whole-body irradiation¹³.

This study was carried out to investigate further the role of host immunity in BCG-mediated suppression of tumour growth by characterising the responses to transplanted rat tumours in athymic mice. Several rat tumours were studied including a carcinogen-induced fibrosarcoma (Mc7) and a hepatoma (D23) both of which exhibit significant immunogenicity, as well as carcinogen-induced and spontaneously arising mammary carcinomas (Sp15, Sp22 and AAF57), which are weakly or non-immunogenic¹². The nude-athymic mice (*nu/nu*), originally described as thymus deficient (MRC Animals Centre, Carshalton), were injected subcutaneously with tumour cells either in Medium 199 or in medium containing BCG (Glaxo freeze-dried percutaneous vaccine; 500 µg moist weight organisms). The tumour cells for these tests were derived from tissue culture lines maintained in Eagle's MEM supplemented with 10% calf serum to ensure that host lymphoid cells were not transferred. It has been shown, for example, that comparable to findings with rat fibrosarcomas¹⁴, tumour cell suspensions prepared from sarcoma Mc7 and hepatoma D23 by trypsin digestion of tumour tissue contain 9-25% glass-adherent macrophages.

Table 2 Growth of second challenge of sarcoma Mc7 cells in mice rejecting a mixed inoculum of Mc7 cells and BCG

Day	First challenge No. of cells in admixture with BCG	Tumour takes	Second challenge		
			Day	No. of cells	Tumour takes in Test Control
0	1 × 10 ⁶	0/4	0	1 × 10 ⁶	2/2 —
			18	1 × 10 ⁶	2/2 3/3
0	1 × 10 ⁶	0/1	0	1 × 10 ⁶	1/1 —

Progressive growths were produced in athymic mice with all five tumours studied using challenge inocula of between 5 × 10⁴ and 10⁶ cultured tumour cells (Table 1). When similar tumour cell challenges were administered in the presence of BCG, however, this generally resulted in complete inhibition of tumour growth. With sarcoma Mc7, for example, challenges of 1 × 10⁶ tumour cells elicited tumours in seven out of eight mice in three separate experiments, whereas only one mouse receiving Mc7 cells together with BCG developed a tumour. BCG treatment also prevented growth of 2 × 10⁶ mammary carcinoma Sp15 and 8 × 10⁴ mammary carcinoma Sp22 cells, although larger tumour cell challenges were not suppressed. None of the tests, however, clearly established suppression of tumour AAF57 by contacting with BCG.

Previous studies^{4,15} in immunocompetent rats with sarcoma Mc7 showed that rejection of tumour cell/BCG mixed inocula produced a specific immune response and this induced rejection of tumour developing at a contralateral site. In this case, rejection was mediated by a specific response against tumour-associated antigens since challenge with another tumour expressing different neoantigens was not rejected. Comparable tests in the athymic mouse model indicate that suppression of sarcoma Mc7 by contact with BCG does not produce inhibition of a challenge with the same tumour either injected simultaneously at a contralateral site or given subsequently after rejection of the tumour cell/BCG inoculum (Table 2).

The implication from these studies is that suppression of tumour growth is not effected by a T lymphocyte mediated response, although the characteristics of the effector mechanisms are still to be elucidated. These may involve a humoral antibody although previous attempts to suppress growth of sarcoma Mc7 with tumour-immune serum in immunocompetent rats have mostly been unsuccessful. There is stronger evidence, however, to suggest that the effects induced by adjuvants such as BCG may be mediated by macrophages as tumour suppression is abrogated in silica-treated rats (R. W. B., and D. G. Hopper, unpublished). Of more practical significance is the observation

Table 1 BCG-mediated suppression of rat tumours in athymic mice

Tumour	No. of cells injected in admixture with BCG*	Tumour takes in	
		Test	Control
Sarcoma			
Mc7	1 × 10 ⁶	0/2†	2/2
	1 × 10 ⁶	0/2	2/2
	1 × 10 ⁶	1/4	4/4
Hepatoma			
D23	1 × 10 ⁵	1/3	3/3
	1 × 10 ⁵	0/3	3/3
Mammary			
carcinoma	2 × 10 ⁵	0/3	3/3
Sp15	1 × 10 ⁶	3/3	2/2
Mammary			
carcinoma	8 × 10 ⁴	0/2	2/2
Sp22			
Mammary	1 × 10 ⁴	0/3	0/3
carcinoma	5 × 10 ⁴	1/3	2/3
AAF57	1 × 10 ⁵	2/2	2/2

*Glaxo percutaneous vaccine (500 µg moist weight BCG organisms).

†None out of two, and so on.

that suppression of tumour growth following contact with adjuvants such as BCG can be achieved in hosts in which immunocompetence is impaired. This may have considerable bearing on the development of clinical studies where immunotherapeutic approaches are often attempted in patients where immunosuppression is already evident as a consequence of surgery, radiotherapy and/or chemotherapy.

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M. V. PIMM
R. W. BALDWIN

Cancer Research Campaign Laboratories,
University of Nottingham,
Nottingham NG7 2RD, UK

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Ir-gene control of immunogenicity of insulin and A-chain loop as a carrier determinant

It is generally believed that the B cells produce antibody against certain antigens after the helper cells recognise a determinant on the carrier part of the antigen. The immune response in mice against several antigens is controlled by Ir-genes which map in the major histocompatibility (H-2) locus of the IXth mouse linkage group. Details of the function of the Ir-genes are still unknown, but it is generally accepted that they control the recognition of carrier determinants^{1,2}.

Although several different antigens are known whose recognition is Ir-gene dependent, the specificity of this recognition mechanism as well as the structure of the carrier determinants remains unclear.

To evaluate the specificity of Ir-gene dependent recognition it is necessary to have a carrier molecule with the following properties. (1) It should be available in highly purified form. (2) Its tertiary structure must be known. (3) It should bear only one carrier determinant. (4) A homologous molecule with small differences at a definite site must be available, in which these alterations do not change the remaining structure of the molecule. (5) Only one of the two homologous molecules must be immunogenic in the strain under investigation.

A comparison of the structure of the two molecules would

yield information about structural requirements for a carrier determinant and about the specificity of the Ir-dependent carrier recognition.

The insulin molecule satisfies all these requirements. It is a small molecule (molecular weight=5,750) so that the number of carrier determinants should be limited for steric reasons. Since the mice tested have their own insulin a further restriction of determinants may be expected. Insulins of different species show small differences in sequence, though the overall structure of the molecule is not altered. The immune response against these carriers is Ir-dependent (unpublished results).

In these experiments the dinitrophenyl(DNP)-derivative of insulin with one DNP-group at lysine in position 29 of the B chain has been used. This derivative was chosen because it can be isolated in a highly purified form by electrofocusing³; hence it can be regarded as free from other contaminating substances which might be highly immunogenic^{4,5}. Another advantage is that the DNP-group as a haptenic determinant

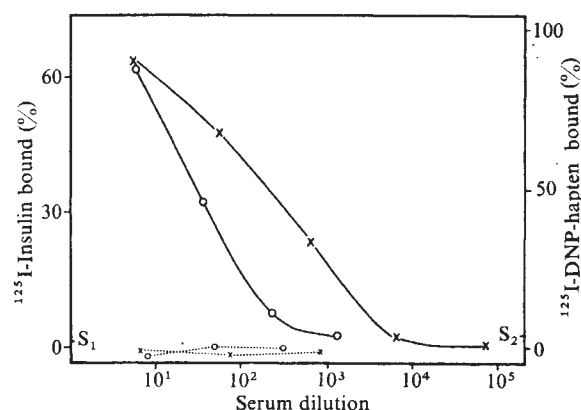


Fig. 1 Response of C57BL mice against DNP-lysine^{B29} insulins. ×, Anti-insulin response, ○, anti-DNP response. Pooled sera were tested. Mice were injected with DNP-lysine^{B29} bovine insulin (solid line) or DNP-lysine^{B29} pig insulin (dotted line). Anti-DNP antibody was measured by the Farr-test using ¹²⁵I-α,N-(4-hydroxyphenylacetyl)-ε-N-2,4-dinitrophenyl-L-lysine as hapten¹¹. Anti-insulin antibody was assayed with ¹²⁵I-bovine insulin by precipitation with 10% polyethyleneglycol¹². The antibody binding capacity (ABC) of a high titre guinea pig antiserum was set as 100%. S₁, S₂, standard deviations of background. Mice were injected with 20 μg antigen on day 1, boosted on day 21 with the same dose in saline and bled on day 30.

serves as an internal control for the processing of the antigen by the antibody-producing B cells. The response pattern is not affected by the introduction of the DNP-group; it also applies to non-substituted insulin, highly purified by electrofocusing, see Table 1.

If pig insulin is used as a carrier the mouse strain BALB/c (H-2^d) gives a high titre of anti-insulin and anti-DNP-antibody, whereas the strains C57BL (H-2^b) and C57BR (H-2^k) show no immune response at all (Fig. 1 and Table 1). If bovine insulin

Table 1 Response of different mouse strains against pig and bovine insulins*

Strain	H-2	Immunogen	Anti-insulin antibody		Anti-DNP antibody	
BALB/c	d	DNP-Lys ^{B29} pig insulin	3.61	(1.22)	3.89	(1.19)
C57BL	b	DNP-Lys ^{B29} pig insulin	<0.1		<0.1	
C57BR	k	DNP-Lys ^{B29} pig insulin	<0.1		<0.1	
BALB/c	d	DNP-Lys ^{B29} bovine insulin	4.51	(1.24)	3.18	(1.22)
C57BL	b	DNP-Lys ^{B29} bovine insulin	3.08	(1.20)	2.7	(1.26)
C57BR	k	DNP-Lys ^{B29} bovine insulin	<0.1		<0.1	
B10.BR	k	DNP-Lys ^{B29} bovine insulin	<0.1		<0.1	
C57BL	b	Pig insulin	<0.1		—	
C57BL	b	Bovine insulin	3.45	(1.31)	—	

*Logarithmic mean of individual titres, s.d. coefficient given in parentheses. Each group 7–10 mice. Experimental details same as in Fig. 1.

is used as carrier, C57BL (H-2^b) is a responder, whereas C57BR (H-2^y) is a non-responder with both insulins. The non-response of B10.BR (H-2^k) is good evidence for the Ir-control of the immune response, since this strain is congenic with C57BL, the only difference being at the H-2 locus.

BALB/c mice (H-2^d) respond to both insulins, so they obviously recognise different determinants, which are not recognised by C57BL mice. The non-response of C57BL against DNP-Lys^{B29} pig insulin may be caused by a deficiency of B cells which can respond to the pig insulin determinants but this is improbable as it has been found that (1) C57BL cannot use pig insulin as carrier for an anti-DNP response and (2) C57BL antibodies against bovine insulin do cross react with pig insulin. The only difference between the two insulins (Fig. 2) is in two amino acids at positions A₈ and A₁₀ in the A chain. These amino acids are located in a small loop which is formed by six amino acids (A₆ to A₁₁) and a disulphide bridge. The sequence of this loop is the same in pig and mouse insulin, whereas bovine insulin contains alanine instead of the more hydrophilic threonine at A₈, and valine instead of isoleucine at A₁₀. The simplest explanation is that the A-chain loop of bovine insulin is the carrier determinant which is recognised by H-2^b mice.

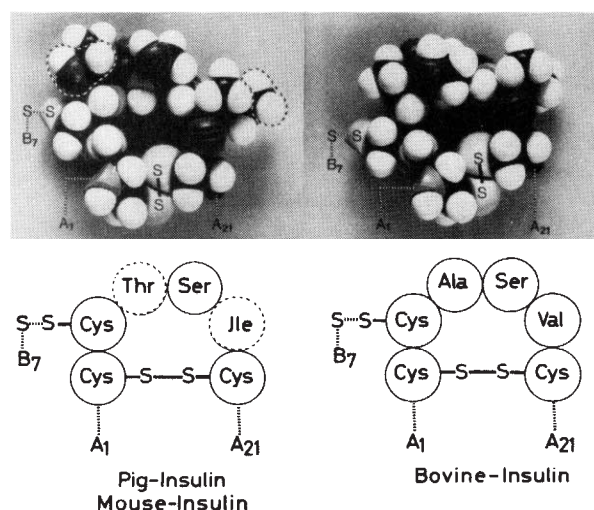


Fig. 2 Structure of the A-chain loop of pig and mouse insulin (left side) which does not act as carrier determinant and the homologous structure (right side) of bovine insulin which acts as carrier determinant in C57BL mice. In the upper picture the marked areas in the 'non-determinant' structure show the -CH(OH)- and the -CH₃ group which are lacking in the determinant.

It may be argued that the exchange of these two amino acids leads to a change in the tertiary structure of the whole molecule, so that the true determinant may not be the A-chain loop itself but another site on the molecule. But this seems to me to be highly unlikely, as this loop is a very rigid structure on the surface of the molecule, further strengthened by a second disulphide bridge at A₇, which links the A chain to the B chain. Therefore, it seems unlikely that changes in this loop affect the overall structure of the molecule⁶. Furthermore there is no difference in biological activity between both insulins.

Our experiments do not show whether the size of the carrier determinant is exactly that of the A-chain loop. Some adjacent parts of the molecule may be necessary or only a part of the loop may act as carrier determinant. It is also possible that, in addition to the loop structure, other sites on the molecule have to be recognised as carrier determinants. In that case, however, their contribution alone would not be sufficient for induction which we have shown in C57BL (H-2^b) mice to be dependent on the presence of the bovine A-chain loop.

It is obvious that the differences between the 'non-determinant' structure (A-chain loop of pig insulin) and the determinant (A-chain loop of bovine insulin) are rather small. There is no charge difference in the two cases and the size and steric arrangement of both structures are very similar. It seems clear that the recognition unit which can discriminate between these two structures must have a high degree of specificity.

It is of special interest to ask whether the specificity controlled by the Ir-genes is reflected in the antibody population, as has been reported⁷. If the sera shown in Table 1 are absorbed with pig insulin coupled with Sepharose, no anti-insulin activity is left in any of them (our unpublished results). Thus, it seems that the high degree of discrimination at the carrier level is not reflected at the level of antibody production, and conversely that the specificity of the antibody is not reflected at the carrier level in C57BL mice. A somewhat similar situation has been described for the hormone glucagon⁸.

Finally, the finding of carrier determinants on insulin and Ir-dependence of their recognition adds same interesting aspects to the problem of insulin therapy in diabetics. Some recent papers claim that pig insulin is non-immunogenic in rabbits if highly purified^{4,5}; others show the opposite⁹. It seems very likely that these discrepancies result from the different genetic background of the experimental animals.

As the insulins of man, mouse and pig have the same sequence in the A-chain loop it seems very likely that the bovine insulin loop can also act as a carrier determinant in man. This assumption is confirmed by the high incidence of anti-insulin antibody in patients treated with the commonly used bovine insulin compared with those treated with pig insulin. It seems reasonable to assume that in man, too, an Ir-gene controls the recognition of this determinant. Until now there has been only weak evidence that such an Ir-gene is located in the HL-A locus of man which corresponds to the H-2 locus in the mouse¹⁰. Experiments designed to establish a correlation of the ability to make antibody against bovine insulin with a certain configuration in the HL-A locus would provide evidence for this assumption.

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KLAUS KECK

Immunology Unit, Division of Biology,
University of Konstanz,
D-775 Konstanz, Germany

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Function of peptide antibiotics in producer organisms

PEPTIDE antibiotics in the producer organisms have been ascribed a wide variety of roles, ranging from evolutionary relics and waste products to elevated control in metabolism (see refs 1 and 2). No hypothesis, however, has yet found

general acceptance. A frequent suggestion is that these antibiotics may have an important regulatory function during the sporulation process of the producer cells^{3,4}. We have recently reported, however, the existence of a bacitracin-negative mutant of *Bacillus licheniformis* which sporulates normally⁵, thus ruling out a direct role for the peptide antibiotic in the formation of spores in this species. To find out whether this mutant is a true non-producer of bacitracin or not, we have compared the bacitracin synthetase complex in this mutant with that of the bacitracin-producing mother strain *B. licheniformis* AL. This suggests that the mutant is not able to produce any bacitracin as a result of a defective enzyme complex.

The non-ribosomal synthesis of the peptide antibiotic bacitracin⁶⁻⁸ is in principle similar to the protein thiotemplate mechanism⁹ for the synthesis of gramicidin S and tyrocidine. The purified enzyme complex from the bacitracin producer *B. licheniformis* AL was resolved in three complementary fractions (A, B, and C) using affinity chromatography. The chromatogram (Fig. 1a) was nearly identical to that obtained using bacitracin synthetase from *B. licheniformis*, ATCC10716 (ref. 10). Each of the three enzyme components activated a different group of the amino acids (measured by the ³²PP_i-ATP exchange reaction) which make up the bacitracin molecules. All three components were necessary for the bacitracin synthesis. Any exchange of corresponding enzyme components

Table 1 *In vitro* synthesis of bacitracin by combined bacitracin synthetase components

Enzyme component	Radioactive incorporation in bacitracin spot (c.p.m.)
A	85
B	120
C	138
AC ₀	115
B ₀	83
A+C	94
A+C+B ₀	1010
B+C	190
AC ₀ +B+C	155
A+B+C	795
AC ₀ +B ₀	90

Bacitracin synthetase from *B. licheniformis* AL and SB319 was fractionated by affinity chromatography as described in Fig. 1. A slower increasing gradient gave the following cross contamination of the fractions A, B, C, AC₀ and B₀ after precipitation with solid (NH₄)₂SO₄ (to 80% saturation) and carrier BSA: Component A contained 8% C; B—4% C; C—15% A; AC₀—3% B₀; and B₀—3% AC₀. The enzyme components were combined (20 µl of each fraction), and the bacitracin synthesising activity was measured (after 15 min incubation at 37°C) using ¹⁴C-L-isoleucine (85,000 c.p.m., specific activity 100 Ci M⁻¹) as described¹⁰. As a result of the experimental separation techniques a certain background count was observed.

from strain AL and strain ATCC10716 did not reduce the amount of bacitracin produced (data not shown).

The chromatogram obtained by fractionating the bacitracin synthetase of the bacitracin-negative mutant *B. licheniformis* SB 319 was different. Using the same affinity chromatography column, the enzyme complex was resolved into two fractions (AC₀ and B₀). Fraction AC₀ activated the same amino acids as the enzyme components A and C of the mother strain, and fraction B₀ activated the same amino acids as enzyme component B (Fig. 1b). There was a marked difference, however, in the degree of the ³²PP_i-ATP exchange reaction with enzymes from the producer and the non-producer. When the non-producer was collected later in growth, the enzyme component AC₀ seemed to be arbitrarily split up. The activation of L-glutamic acid was performed by a component dissociated from AC₀.

The combination of enzyme fractions from the producer

and the non-producer showed that enzyme component B₀ of the non-producer was able to participate in the bacitracin synthesis of the producer strain instead of enzyme component B, but enzyme component AC₀, when combined with intact enzyme component B from the producer, was not able to do so (Table 1). Similar results were obtained when enzyme components A, B, and C from ATCC10716 were used. This suggests that the bacitracin synthetase of the bacitracin-negative SB319 was defective. This defect seems to influence the activation sites for the amino acids and the affinity between the

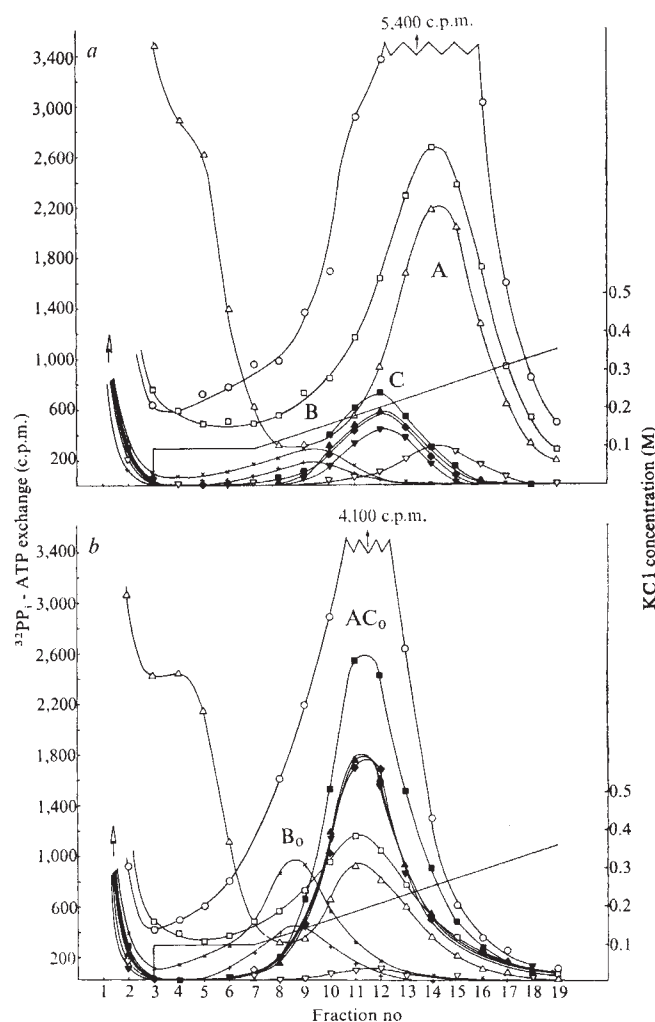


Fig. 1 Fractionation of bacitracin synthetase by affinity chromatography. *B. licheniformis* AL (a) and SB319 (b) were grown in the medium of Haavik and Thomassen⁵. The cells were collected at an absorbance of 4.5 measured in a Spectronic 20 spectrophotometer at E_{630} , lysed with lysozyme, and the enzyme was partially purified by streptomycin sulphate precipitation and ammonium sulphate precipitation (to 55% saturation) as described by Frøyskov and Laland⁸. The 55% (NH₄)₂SO₄ fraction obtained from 250 ml culture was applied to an affinity chromatography column (3.0 × 1.6 cm) prepared from 2 g Sepharose 4-B (Pharmacia Fine), and 3, 3'-diaminodipropylamine, with L-leucine as ligand as described⁸. The column was eluted with 0.05 M potassium phosphate buffer (pH 7.5) containing 0.2 M MgCl₂ and 0.1 mM dithiothreitol (60 ml h⁻¹). A KCl gradient (0.1–0.4 M) was added to the buffer, and ³²PP_i-ATP exchange activity dependent on the L-amino acids isoleucine (○), cysteine (□), leucine (△), glutamic acid (▽), lysine (+), ornithine (×), phenylalanine (◇), histidine (▲), aspartic acid (■), and asparagine (◆) was measured in 0.2 ml aliquots of each fraction (6.5 ml) as described¹⁰. Total amount of radioactivity in each incubation mixture was 120,000 c.p.m. The radioactivity (40–50 c.p.m.) in control tubes (without any amino acids) was subtracted. The signs A, B, C, AC₀, and B₀ indicate the enzyme fractions used in further experimentation.

enzyme components corresponding to A and C of the producer.

Previously⁵, neither external nor internal bacitracin were detected, yet the mutant SB319 sporulated normally, indicating that bacitracin has no regulatory role during sporulation. With reports of other antibiotic-negative mutants able to sporulate^{11,12}, evidence seems to be accumulating against a possible connection between peptide antibiotics and sporulation of producer organisms.

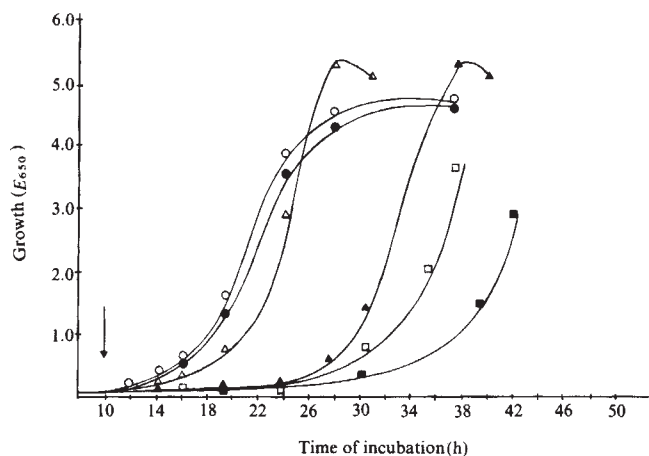


Fig. 2 Growth of *B. licheniformis* strains at different cultural conditions. Growth of strain SB319 in the medium with 0.0001 g $\text{MnSO}_4 \text{l}^{-1}$ (○); with 0.1 g $\text{MnSO}_4 \text{l}^{-1}$ (△); with 0.1 g $\text{MnSO}_4 \text{l}^{-1}$ and 50 IU bacitracin ml^{-1} added (□). Arrow indicates time of addition. The corresponding growth curves of strain AL are marked ●, ▲, ■, respectively. Growth was measured as E_{650} using a Specronic 20 spectrophotometer. Growth occurred in 500 ml Erlenmeyer flasks incubated at 37 °C at about 360 r.p.m. on a New Brunswick rotatory shaker (Model G-53). The defined medium used had the following composition (g l^{-1}): L-glutamic acid (15.0), L-leucine (2.0), L-histidine (0.2), L-alanine (0.2), citric acid (1.0), KH_2PO_4 (0.1), K_2HPO_4 (0.1), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.2), $\text{FeSO}_4 \cdot (0.01)$, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (varied between 0.0001 (low) and 0.1 (high) as noted in the text), pH 7.0. The inoculum consisted of spores¹³.

The production of peptide antibiotics after growth is assumed to be a fundamental property of the microbial cell, and thus the search for their function has been focused on the stationary phase metabolism²⁻⁴. We have reported, however, that the lag in antibiotic production in relation to growth may be pH-dependent^{13,14}. Peptide antibiotics may therefore normally be synthesised, and may also function throughout active growth. The observations that tyrocidine and linear gramicidin production under certain conditions are paralleling growth¹⁵, that polymyxin E production occurs in the log phase of growth¹⁶, and that penicillin¹⁷, tyrothricin¹⁸, and gramicidin S (ref. 19) can be produced in continuous culture, support this view.

Both the producer and the non-producer strains of *B. licheniformis* show similar sporulation and also identical reactions in standard biochemical tests⁵. To determine the function of bacitracin during growth, the two strains were grown at several different cultural conditions. At present only a few significant differences between the producer and the non-producer have been discovered. The bacitracin-negative mutant showed a markedly increased resistance to high concentrations of Mn^{2+} . In media with low manganese both strains showed almost identical growth (Fig. 2).

Active manganese transport in *B. subtilis* has recently been described. Too high concentrations of manganese in the medium inhibited growth as a result of an efficient uptake of the ion^{20,21}. Our bacitracin-negative mutant, SB319, was only slightly inhibited at manganese concentrations which strongly inhibited the producer strain AL, indicating that the manganese uptake of the mutant was markedly less efficient than that of the producer. If bacitracin participates in the transport of

Mn^{2+} , this difference between the two strains could be explained. Furthermore, the addition of bacitracin to the bacitracin-negative strain should eliminate this difference. Indeed, when bacitracin was added to the non-producer, growth was markedly inhibited in media with high manganese (Fig. 2). Adding bacitracin to the same culture grown in low manganese showed no effect. The addition of bacitracin to the producer strain increased the inhibitory effect of high manganese concentration (Fig. 2).

The possibility that peptide antibiotics normally act as carriers of ions through membranes in the producer organisms has been suggested^{22,23}. This agrees with the strong metal binding properties of peptide antibiotics^{24,25}, and their ability to modify membrane permeability²⁶. Since the bacitracin-negative mutant, SB319, is able to grow without producing any bacitracin, this mutant must take up manganese by some other means. Our results indicate, however, that bacitracin is able to influence this manganese transport by increasing the efficiency of the uptake of the ion. By depriving the non-producer and the producer strains of manganese by adding EDTA to the medium, the bacitracin-negative mutant was markedly inhibited whereas the bacitracin producer grew well. By adding bacitracin or excess manganese to the EDTA-inhibited mutant, growth was promoted (H.I.H., unpublished). This indicates that bacitracin production may be necessary in environments with a very low manganese content.

The antimicrobial effect of bacitracin may be an interaction with the cytoplasmic membrane of susceptible organisms²⁶. This effect is dependent on the presence of certain divalent cations such as Mn^{2+} (ref. 27), the function of which may be to promote the cell-antibiotic interaction²⁶. A similar mechanism may operate in the producer strain: bacitracin may increase the uptake of manganese by promoting the interaction between manganese and the cytoplasmic membrane.

In conclusion, we have shown that the bacitracin-negative mutant (SB319) has only a partially functional bacitracin synthetase complex. This mutant is probably not able to produce any molecules of bacitracin. A comparison of the producer and non-producer of bacitracin in different cultural conditions indicates that the peptide antibiotic bacitracin participates in the transport of manganese through the bacterial membrane. Further experimentation will be necessary to determine whether this is causal or coincidental.

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HANS I. HAAVIK
ØYSTEIN FROYSHOV

Department of Research and Development,
A/S Apothekernes Laboratorium for Special præparater,
Oslo, Norway

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Improvement of antisera against polypeptide hormones

THE sensitivity of immunoassays is probably decreased by endogenous antigenic material blocking some of the antibody binding sites¹. To remove antibodies generated against polypeptide hormones from endogenous antigenic material derived from the immunised animals, we took advantage of the capacity of sodium iodide to break the antigen-antibody bond.

Antisera against lys₈-vasopressin (Ferring, Malmö) and val₅-angiotensin II (Ciba, Basel) were generated in rabbits with repeated intramuscular injections of immunogen coupled to albumin². Angiotensin was labelled with ¹²⁵I (IMS 3, Amersham) as described previously². In the case of arg₈-vasopressin (Ferring, Malmö) the metabisulphite step was omitted. Only monoiodinated material was used, as confirmed by isoelectric focusing. Specific activity for angiotensin II was 450 $\mu\text{Ci } \mu\text{g}^{-1}$, and for arg₈-vasopressin 650 $\mu\text{Ci } \mu\text{g}^{-1}$.

Antisera were diluted at ratios of 1:700-1:2,000 with 0.9% NaCl and treated with 2M NaI (final concentration) at room temperature for 2 h, then dialysed for 48 h at +4 °C against

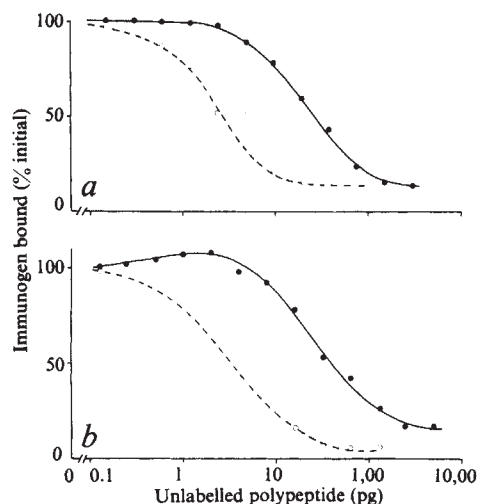


Fig. 1 Increase in sensitivity. *a*, Dose-response curve for val₅-angiotensin II (mean of five experiments) before (●) and after (○) treatment of the antiserum with NaI. *b*, Dose response of arg₈-vasopressin (mean of eight experiments) shows a paradoxical increase in binding^{5,6} before (●) and a shift to the left with disappearance of the paradoxical binding after (○) iodide treatment.

0.9% NaCl. The diluent for binding studies with vasopressin was 0.15 M phosphate buffer (pH 7.5), containing human serum albumin (Kabi, Stockholm) 1 mg ml⁻¹, Na₂-EDTA 10 mM, L-cysteine 20 mM neomycin sulphate (Upjohn) 0.1 mM; and for angiotensin the same buffer with OH-quinoline 1.5 mM (Merck) instead of L-cysteine.

The procedure of sodium iodide treatment and subsequent

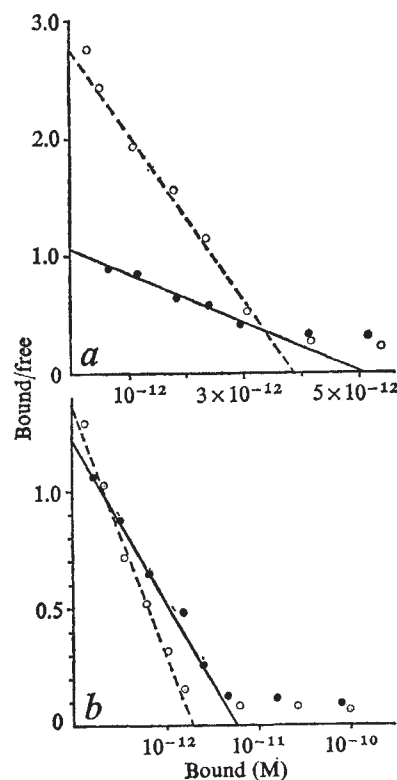


Fig. 2 Increase in affinity. Scatchard plots⁴ for antisera to angiotensin II (*a*) and lys₈-vasopressin (*b*) before (●) and after (○) NaI treatment (mean of three experiments). The apparent affinity constants³ were $2.1 \times 10^{-13} \text{ M}^{-1}$ and $7.1 \times 10^{-13} \text{ M}^{-1}$, respectively, for untreated and treated antiserum to lys₈-vasopressin, and $1.7 \times 10^{-13} \text{ M}^{-1}$ and $5.9 \times 10^{-13} \text{ M}^{-1}$ respectively, for angiotensin II.

dialysis increased the sensitivity of the dose-response curves for arg₈-vasopressin and angiotensin II by factors of 12.1 and 10, respectively (Fig. 1). This increase in sensitivity could not be explained as a dilution effect because conditions were designed to give maximal sensitivity³ both before and after NaI treatment of the antiserum. Scatchard plots⁴ (Fig. 2) indicated modification of the apparent affinity constants of the antisera with iodide treatment, and some loss of total binding capacity. We conclude that this resulted from the unmasking of high affinity antibody binding sites blocked by endogenous antigenic material.

The dose-response curves for unlabelled analogues of angiotensin II and vasopressin, taken as a measure of specificity, were shifted (Fig. 3). This resulted in an increased specificity for val₅-angiotensin II compared with the 2-8 heptapeptide and 3-8 hexapeptide fragments. In the vasopressin assay system, iodide treatment of the antiserum caused the highest increase in sensitivity for unlabelled lys₈-vasopressin compared with arg₈-vasopressin and pressinoic acid. In other words, the assay became more sensitive to the hapten (lys₈-vasopressin) actually used to immunise rabbits against vasopressin. We believe that the changes in apparent specificity observed resulted from the unmasking of binding sites with a high affinity for this molecule.

The paradoxical binding of vasopressin (that is, increase in bound label with low doses of unlabelled polypeptide), which has been used by others in highly sensitive radioimmunoassays for ACTH (ref. 5) and LH-releasing hormone⁶, disappeared with sodium iodide treatment (Fig. 1). Thus, paradoxical binding may be caused by endogenous antigen interfering with the binding of iodinated polypeptide. On the other hand, iodide treatment may induce changes in the steric configuration of antibody molecules resulting in a loss of paradoxical binding.

Our data suggest that any procedure for removal of endogen-

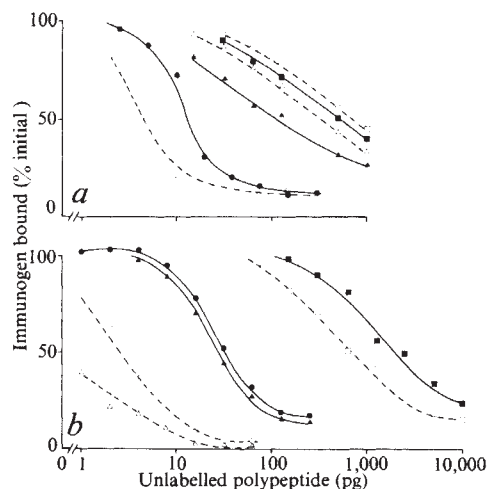


Fig. 3 Modification of specificity. *a*, Dose response curve of val₅-angiotensin II (●) radioimmunoassay is shifted to the left (○); curves of 2-8 ileu₅-heptapeptide (▲) and 3-8 ileu₅-hexapeptide (■) are shifted to the right (△, □) after sodium treatment, resulting in a decreased cross reaction with heptapeptide and hexapeptide. Mean of two experiments. *b*, Curves of lys₈-vasopressin (●), arg₈-vasopressin (▲) and pressinoic acid (Ferring, Malmö, ■) were shifted to the left after iodide treatment (○, △, □). The result was increased sensitivity for lys₈-vasopressin compared with arg₈-vasopressin, and a decreased cross reaction with pressinoic acid after iodide treatment. Mean of three experiments.

ons antigenic material which causes minimal damage to the antibodies should be considered when adequate sensitivity of immunoassays cannot be achieved with known designs of competitive protein binding assay³. The treatment of antisera with chaotropic substances such as sodium iodide may also result in a modification of antiserum specificity.

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FREJ FYHRQUIST
MARJA WALLENIS

Unit of Clinical Physiology,
Minerva Institute for Medical Research,
PO Box 819, Helsinki 10, Finland

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Conservation of primary structure in 16S ribosomal RNA

THERE is no doubt that the large ribosomal RNAs play specific roles in ribosome function¹. Yet the thrust of experimentation during the past 5 years indicates clearly that the biologist tends to view these roles as structural, function being reserved by and large for the ribosomal protein components^{2,3}. Whether or not this prejudice can be maintained remains to be seen. In any case, the existence of an approximate sequence for a 16S rRNA provides a basis for detailed characterisations of this molecule^{3,4}. Here we begin to define 16S rRNA function by localising the major conserved regions in the molecule through a comparative analysis of 27 prokaryotic 16S rRNA primary structures.

Comparative sequencing has been used effectively to

investigate the structure and function of a number of small macromolecules. Although this approach is not feasible for the larger ribosomal RNAs, partial sequencing techniques can be used effectively. One can, for example, characterise a large RNA by comparative cataloguing^{5,6}; that is, completely delineate the products of T₁ ribonuclease digests of RNA species from various organisms. In practice, this can be done using present techniques⁷ with only a few oligonucleotides eluding sequencing.

When comparing two such oligomer catalogues, a prime consideration is the size for which oligomer sequence coincidence implies true primary structural homology. Statistical considerations⁵ indicate that coincidence among oligonucleotides of length six or more (pentanucleotides are marginal) provides strong evidence for primary structural homology in a sequence of 1,600 nucleotides. When three or more catalogues are considered, the continuous appearance of any oligonucleotide of length five or more increases the probability of true homology, as the prospect of multiple chance coincidence shrinks rapidly as the number of catalogues considered increases.

During the past few years, complete ribonuclease T₁ catalogues have been developed in our laboratory for the 16S rRNAs of a wide range of prokaryotes, including Enterobacteriaceae⁸⁻¹⁰, Spirillaceae¹⁰, Cyanophyta¹¹ and Athiorhodaceae¹², and there are unpublished results from this laboratory for Bacillaceae, Brucellaceae, Achromobacteraceae, Pseudomonadaceae and Lactobacillaceae. It is now appropriate to consider the extent to which each of the characteristic oligomers (pentanucleotides and larger) of *Escherichia coli* 16S rRNA is conserved across phylogenetic lines. These 114 oligonucleotides are shown in Table 1 together with the number of organisms in which each has been found. They are arranged in their reported order of occurrence⁴ in the 16S rRNA sequence and numbered alternately so that nomenclature adjustments for minor sequence revisions can be accommodated readily.

When the relative occurrence data of Table 1 are depicted graphically (Fig. 1), the pattern of conservation characterising 16S rRNA primary structure is displayed vividly. The oligomers that appear in more than half the organisms screened are considered to be conserved, while those found in at least 24 of the 27 organisms are termed universal. Figure 1 suggests nine possible concentrations of conserved and universal oligomers: (1) oligomers 217-223, (2) 185-207, (3) 167-177, (4) 153-159, (5) 127-137, (6) 107-113, (7) 75-83, (8) 41-51 and (9) 31-35. Although the conserved oligomers are divided evenly between the two halves of the sequence, the universals are not (19 out of 27 are in the 3' half). Thus, as might be expected, six of the nine highly conserved regions are in the 3' half of the molecule. Indeed, the 3' half of the molecule seems to alternate quite regularly between regions of extremely high and low conservation.

The most prominent region of conservation, No. 2, is very close to the 3' end of the molecule, between oligomers 185 and 207. The 12 large oligomers in this region together encompass approximately 100 nucleotides. The final oligomer in this grouping, U^{*}CACCAUG, is present in only 14 organisms. The clearly related sequences, UCA^{*}ACCAUG and UCA^{*}ACCACG (asterisks indicate post-transcriptionally modified nucleotides), however, account for 12 of the remaining organisms.

Several additional regions deserve further comment. Region No. 1, the 3' terminal region, is excised intact by colicin E3 (refs 13 and 14). In a large group of prokaryotes, this region contains the kasugamycin-sensitive sequence¹⁵ which may provide a clue to the region's functional significance. Region No. 6 comprises merely three oligomers but may be somewhat larger as it lies immediately adjacent to a gap in the sequence. Three oligomers not currently placed that might appear in this gap are the highly conserved CAAACAG and the possibly universal copies of two pentamers, AUUAG and AAAUG (Table 1 and footnotes a and k).

In addition to locating the conserved regions, it is important

Table 1 Phylogenetic conservation of oligonucleotides in *E. coli* 16S ribosomal RNA

Oligomer composition	No. of occurrences	Comments	Oligomer composition	No. of occurrences	Comments
1 p AAAUUG	4		115 ACUUG	7	
3 AUC AUG	12	B	117 CCUUG	5	
5 CUC AG	26/8	Two copies	119 CCUUG	15/10	Two copies
7 CUU AAC CAUG	11		121 (g) CUU ACG	19	
9 UAA CAG	7	B	123 UUA AG	26/13/4	Three copies
11 CUUUG	4		125 (h) ACCCG	8	
13 UAAUG	17		127 UUA AAA CUCAAUG	14	
15 AAACUG	21		129 AAUUG	26	
17 AUAA CUACUG	10		131 CACAAG	27	
19 AAACG	13		133 AUG*	11	
21 CUAAUACCG	21		135 CAACG	26	
23 CAUAAACG	3		137 UUUA AUUUG	25	
25 ACCUUG	7		139 AACCUUACCG	3	
27 CCUUG	3		141 CUUUG	18	May be two copies
29 CCUUG	20		143 (i) ACCAUACG	0	
31 CCCAG	25/14	Two copies, B in one case	145 UUUCAG	1	
33 (a) AUUAG	17		147 CUUCG	6	
35 UAACG	5		149 ACCCG	8	
37 CUCACCUAG	7		151 (j) UCCCG	27	Second copy, if present, may be here. No. 151 is not shown on Fig. 1
39 AUCCCUAG	6/0	May be two copies			
41 (b) ACCAG	24		153 (k) AAAUG	25/12	
43 CCACAUG	17/12	Multiple copies: at least 2, possibly 3	155 UUAAG	26/13/4	A, B, C
45 (c) AACUG	27/4		157 (j) UCCCG	27	A, B, C
47 (b) ACACG	10		159 C* A ACG	27	B
49 UCCAG	23		161 CAACCCUUAUCCUUG	6	
51 ACUCUACG	14		163 AAUCUAAAG	5	
53 AAUAUUG	10		165 UUA A ACG	3	B
55 CACAUG	14		167 UCAAG	21	
57 CAUG	15/10	Two copies	169 UCAUACG	14	
59 CCUUG	27/17	Multiple copies, at least 2, possibly 3	171 (l) CCUUAACG	14	
61 UAAAG	2		173 (b) ACCAG	6/0	May be two copies
63 UUAUAUCCUUG	1		175 (m) CUACACAACG	22	
65 CUCAUUG	3		177 CUACAAUG	26	
67 UUA CCG	13/16		179 CAUACAAG	2	
69 (d) CCAAG or CACCG	6		181 ACCUCG	4	
71 UACUUUCAG	12		183 ACCUCAUAAAG	4	
73 CUAA CUCG	27		185 CAACUCG	25	
75 (e) G* CCG	25		187 ACUCCAUG	8	
77 UAAUACG	13	B	189 AAUCG	24	A, B
79 UAAUUCG	14		191 UAAUCG	27	B
81 AAUAUCG	27/17	Multiple copies, at least 2, possibly 3 three copies	193 AUCAG	26	
83 UAAAG	26/13/4		195 (d) CACCG or CCACG	16/13	
85 UUAAG	6		197 AAUACG	26	
87 AAUCCCG	17/12	Two copies	199 UUCG	26	
89 CUCAACCG	3		201 CCUUG	23	C
91 (c) ACUG	6		203 UACACAACCG	25	B, C
93 CAUCG	3		205 CC* CCG	27	
95 AUUCG	5		207 UC* ACACCAUC	14	
97 UUCG	13		209 AUUCAUG	9	A
99 AAUCCAG	11	B	211 CAAAAG	9	A
101 AUCUG	11	B	213 CUUAACCUUG	8	A
103 AAUAACG	26/8	Two copies	215 CUUAACCAUUG	6	A
105 (f) CCCCUG	26	A, B	217 U* AACAAAG	27	A, C
107 CUCAG	22	B	219 (g) UAACCG	19	
109 AUACCCUG	26		221 A* A* CCUG	14	A, B
111 UCCACG	26		223 (a) AUCACCU (U, C C) UA _{OH}	13	A
113 UAAACG	26		(p) CAACACG	23	
			(p) ACCAAAG	6	

The first column lists the 114 ribonuclease T₁ oligonucleotides ($\geq$ pentamers) in the order in which they have been placed by Ehresmann *et al.* in the 16S rRNA sequence unless indicated otherwise in the footnotes⁴. The oligomer sequences used, however, are those determined by Uchida *et al.*⁷ rather than those reported by Fellner *et al.*¹², unless otherwise indicated in the footnotes. The second column indicates the number of organisms in which the given oligomer has been found. The final column indicates by (A) those oligomers which have been found to be excised by ribonuclease T₁ from intact 30S subunits (T.U. and C.R.W., unpublished), by (B) those oligomers which have been found to be substituted by kethoxal at the 5' guanosine and by (C) those substituted at the 3' guanosine¹⁶.

Notes to Table 1

(a) This oligomer is found in two copies in *E. coli* and most 16S rRNAs from other Enterobacteriaceae and Spirillaceae, but in one copy in many other organisms. Since Ehresmann places only one copy, it is not clear that position 33 is the correct location for the universal copy. If this universal oligomer is ultimately placed elsewhere, conserved region No. 9 will no longer exist.

(b) Ehresmann locates two copies of ACCAG (No. 41 and No. 173), one copy of ACACG (No. 47) and no copies of CACAG. Previously Fellner reported one of each. Uchida found 1–2 copies of ACACG, only one copy of ACCAG and no CACAG. Thus these three oligomers are currently ambiguous and are treated as such on Fig. 1.

(c) This oligomer has been reported as present in 2–3 copies by Uchida and 3 copies by Fellner. Yet only two have been located by Ehresmann (No. 45 and No. 91) and one of these (No. 91) is considered to be fractional.

(d) Due to the inability of Fellner and Ehresmann to distinguish between CACCG and CCACG, these two oligomers (No. 69 and No. 195) are not uniquely placed at this time and are thus represented by open bars on Fig. 1.

(e) The sequence previously listed by Uchida was that obtained by Fellner. This oligomer has now been independently examined and the sequence is found to be as reported here (L. Z. and C. R. W., unpublished).

(f) This oligomer has been re-examined with more powerful techniques and the sequence given here is taken to be correct rather than that previously reported by Uchida.

(g) These two isomers are placed here in a fashion consistent with the results of Santer and Santer²⁴. However, Ehresmann suggests the opposite placement.

(h) No evidence for this oligomer has been found in our preparations of *E. coli* 16S rRNA (see Uchida). Although it is found in eight other organisms, it is not found in any Enterobacteriaceae or Spirillaceae 16S rRNA so far examined. The oligomer is thus indicated on Fig. 1 by an open bar.

(i) This sequence and what seems to be the earlier version of it, CAUCACG, reported by Fellner has not been found in *E. coli* by Uchida, nor has either been found in any other organism. Its position is indicated on Fig. 1 as a fractional solid bar.

(j) Two copies of this oligomer are reported to be very close to each other in this area of the sequence. The sequencing is, however, rather incomplete here, and Ehresmann indicates the first copy is questionable. Although Uchida reports 1½ copies, no other Enterobacteriaceae or Spirillaceae is known to have two copies. Thus only one copy (No. 151 is omitted) is included in Fig. 1. Furthermore, the order of No. 155 and No. 157 is that suggested by Noller¹⁶ rather than that of Ehresmann.

(k) Fellner reports only one copy of this oligomer and Ehresmann locates only one. Uchida, however, reports 2 and every member of the Enterobacteriaceae and Spirillaceae so far examined has been found definitely to contain 2. Thus this oligomer has been represented as an open bar on Fig. 1.

(l) This oligomer represents a minor correction to the sequences given by Uchida (C. R. W., unpublished).

(m) This oligomer was previously unsequenced by both Uchida and Fellner and is now known to have the sequence indicated (C. R. W., unpublished).

(n) The sequence previously listed by Uchida for this oligomer was that reported by Fellner. This oligomer has now been independently examined and the sequence is found to be as reported here (C. R. W., unpublished).

(o) Although the 3' terminal oligonucleotide has not yet been fully sequenced in this laboratory, it is known to vary across phylogenetic lines. Certainly the first five and probably the first seven nucleotides, AUCACCU... are found in all but one case, and the remaining bases in all cases are a mixture of uracils and cytosines (C. R. W. *et al.*, unpublished).

(p) These two oligomers have not yet been located in the *E. coli* 16S rRNA sequence by Ehresmann. They are, however, reported by both Uchida and Fellner.

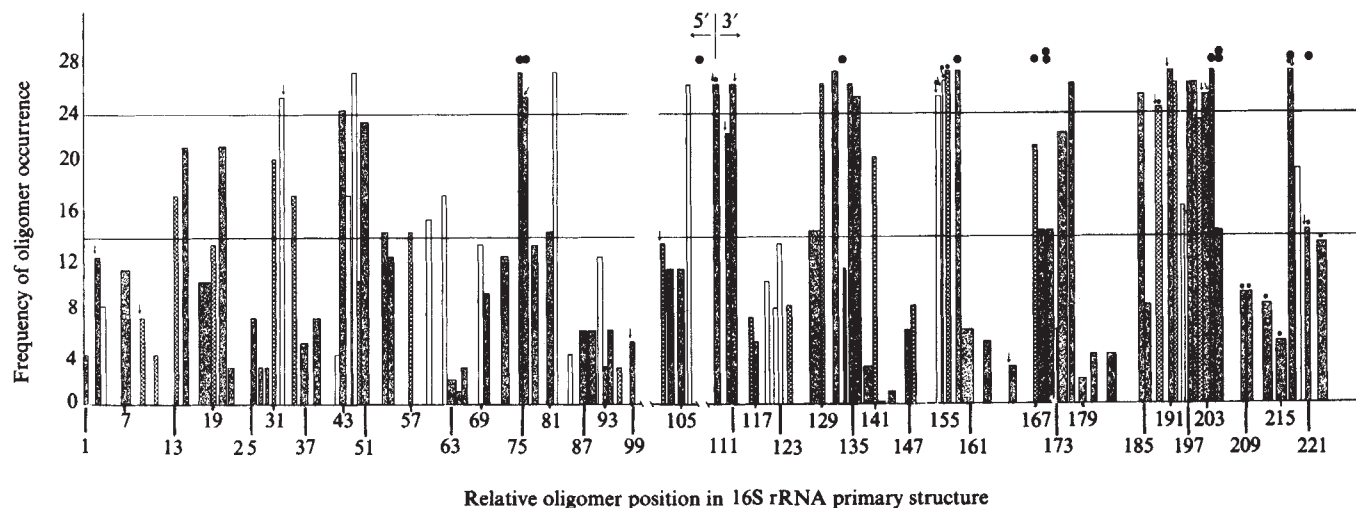


Fig. 1 Distribution of phylogenetic conservation in 16S rRNA. Each bar represents one of the characteristic oligonucleotides of the *E. coli* 16S rRNA sequence. The ordinate shows the number of organisms which have been found to contain that oligomer and the abscissa indicates their exact position in the sequence of Ehresmann *et al.*⁴; numbers are those given in Table 1. The width of the bars is proportional to the size of the oligomers. The known gaps in the sequence are indicated by (—/—) on the abscissa. The solid bars designate unique oligomers containing six or more bases or a post-transcriptionally modified base. The cross hatched bars represent unique oligomers of length five. The open bars represent the most consistent placement of those oligomers which are not unique; their placement must be considered highly tentative. Arrows above the bars indicate the points of kethoxal substitution, positioned so as to indicate whether the substituted guanosine is at the 5' or 3' end of the oligomer¹⁸. The small dots indicate known points where T_1 ribonuclease can remove an oligonucleotide from the intact 30S subunit. The large dots indicate the location of post-transcriptionally modified bases that are found in *E. coli* or other 16S rRNAs.

to consider which of them are exposed on the 30S ribosomal subunit. This problem can be approached in a number of ways: (1) by determining points of nuclease attack on the 30S subunit; (2) by locating post-transcriptionally modified nucleotides (since an RNA segment must be exposed at some time during ribosome assembly to allow attack by the modifying enzyme), and (3) by determining points of attack with chemical modifying reagents. All these techniques have been used, and the results are included in Fig. 1.

The location of post-transcriptional modifications as defined by *E. coli* oligomers and those found in related sequences of the other 26 organisms correlate completely with the conserved segments. Each of the six conserved regions in the 3' half of the molecule and the adjacent one in the 5' half of the molecule contains modifications in some if not all organisms. No modification is found in an unconserved region of the molecule. Kethoxal modifications of the intact ribosome have been made and identified¹⁶. These correspond strongly with the highly conserved regions Nos 2, 4 and 6 which contain respectively 2, 4, 3 and 3 kethoxal attack points. Attack points found by T_1 ribonuclease digestion of intact 30S subunits (T. U. and C. R. W., unpublished) also follow this general pattern. This last study indicates that oligomers 209–223 are especially susceptible to attack. Santer and Santer¹⁷ found oligomers 207–217 to be susceptible in a similar study.

The ribosomal protein-binding sites so far characterised seem by and large not to coincide with the conserved regions^{18–21}. All the primary binding proteins (with perhaps one exception) can be localised in the 5' half of the molecule. There does exist, however, a ribonucleoprotein subparticle containing proteins S7, S9, S13 and (usually) S14 (ref. 22), that includes some oligonucleotides from region No. 2 (its 5' portion). This in itself is not proof that region No. 2 actually contains a protein-binding site.

If this apparent negative correlation proves entirely correct it raises an interesting problem. Since a functional 30S ribosome chimera can be constructed from *E. coli* 30S proteins and *B. stearotherophilus* 16S rRNA²³, one would assume homology of the protein-binding sites in the two organisms. The apparent contradiction would be resolved if homology were in this instance to be primarily in secondary or tertiary structure and so on. There is a precedent for such an interpretation in the 5S rRNA. This molecule is functionally interchangeable between the

above two organisms²⁴, yet it is likely that its ribosomal protein-binding sites are again not in the regions of highest primary structural conservation^{25, 26}.

The results reported here show the extent to which each of the characteristic ribonuclease T_1 oligomers of *E. coli* 16S rRNA is conserved. The universal and highly conserved oligomers cluster, falling into not more than nine regions. These groupings are not uniformly distributed throughout the molecule, but rather are concentrated in the 3' half where they alternate regularly with regions of low conservation. At least seven of the regions are located on the surface of the 30S particle (at some stage), including all of those in the 3' half of the molecule. These results strongly suggest that important functional features of the 16S rRNA are located in the 3' half of the molecule. Since little or no correlation exists between these conserved regions and known ribosomal protein binding sites, the implication is strong that large areas of the RNA are directly involved in ribosomal function.

CARL R. WOESE
GEORGE E. FOX
LAWRENCE ZABLEN
TSUNEO UCHIDA*
LINDA BONEN
KENNETH PECHMAN
BOBBY J. LEWIS
DAVID STAHL

Departments of Microbiology and Genetics and Development,
University of Illinois,
Urbana, Illinois 61801

Received November 15, 1974; revised January 13, 1975.

*Permanent address: Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo.

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Formation of triple-stranded bovine DNA *in vitro*

EVIDENCE for a triple-stranded complex of polynucleotides was reported for homopolymer RNAs by Felsenfeld *et al.*¹ in 1957. They detected a drop in absorbance at 260 nm for a mixture consisting of two parts polyadenylic acid (poly(rA)) and one part polyuridylic acid (poly(rU)), during a continuous variation mixing study of the two homopolymers. Addition of 0.1 M MgCl₂ produced the greatest triple-strand formation with a minimum absorbance at 67 mol % poly(rU), 33 mol % poly(rA)². Since this early work, many triple-stranded complexes have been identified, including combinations of homopolymers of deoxyribonucleotides and ribonucleotides; Riley *et al.*³ reported a triple strand formed by the homopolymers dA: dT: dT. They also reported the caesium sulphate buoyant densities of various double-stranded and triple-stranded homopolymers. In all cases, the triple-stranded homopolymer had a greater buoyant density than the analogous double-stranded homopolymer. We now report evidence for the formation of triple-stranded bovine DNA *in vitro*.

We used a magnesium salt of bovine DNA, which was prepared from bovine spleen by substituting MgCl₂ for the sodium chloride-sodium citrate medium (SSC) in a standard DNA preparation procedure⁴. Sodium xylene sulphonate (Naxonate G) was added to precipitate protein. The MgDNA so prepared was purified by reprecipitating with cold 2-propanol three times, and the final precipitate was dried by washing first with ethanol and then with acetone. When the purified MgDNA was dissolved in 5 × 10⁻⁴ M MgCl₂, the usual DNA

absorption spectrum was obtained with a maximum at 260 nm, the ratio of A_{260} to A_{235} was 2.28. The temperature-absorbance curve for MgDNA, dissolved in deionised water, is shown in Fig. 1; T_m for MgDNA was 85-87 °C; the return on cooling was about 50%, and the hyperchromicity was 43%. This contrasts with the T_m for spleen NaDNA in deionised water, which was 71 °C. In both cases, the initial absorbance at 260 nm was closer to 0.95. The melting profile for MgDNA was steeper than that for NaDNA, indicating extensive stabilisation of the DNA double helix by Mg(II). The ratio of Mg(II) to DNA(P) was 0.518, indicating one magnesium per two nucleotide phosphates. MgDNA was also stable to hydrolysis by pancreatic DNase I in the absence of added Mg(II): addition of Mg(II) to the above solution resulted in rapid degradation of the DNA⁵. DNase I requires free Mg(II) for activity; thus very little of the Mg(II) in MgDNA was available.

Solutions of single-stranded NaDNA were prepared by heating to 95 °C and cooling quickly, solutions of native bovine NaDNA in deionised water. This process was repeated, until the melting curve of the resulting solution showed no rise in absorbance when heated.

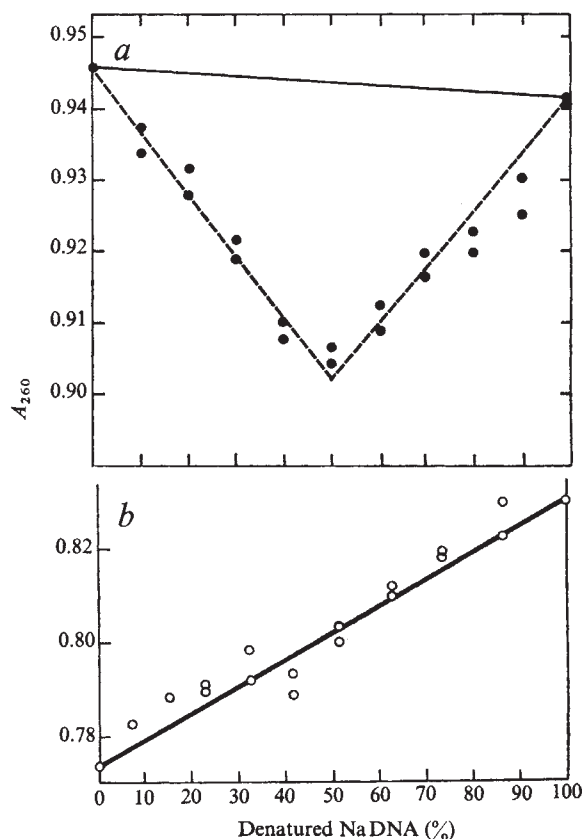
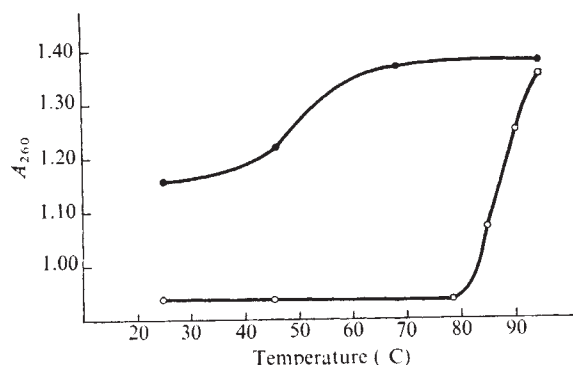


Fig. 2 Continuous variation mixing curves for mixtures of native bovine MgDNA and denatured NaDNA. The unbroken line is the expected curve for no interaction, % denatured NaDNA is mol %, and the absorbance was measured at 260 nm. a, MgDNA and NaDNA in 0.1 M MgCl₂; b, MgDNA and NaDNA in deionised water.

Fig. 1 Temperature-absorbance curves for MgDNA in deionised water. Absorbance was measured at 260 nm. ○, Heating curve; ●, cooling curve.



Continuous variation mixing curves, as used by Riley³ and others, were made to determine the interaction of MgDNA and denatured NaDNA. Figure 2 shows mixing curves for the two DNAs: (a) in the presence of 0.1 M MgCl₂ and (b) in water only. The unbroken line is the expected curve for no interaction. (0.1 M MgCl₂ was found to be the optimum concentration of MgCl₂ in our studies, and for homopolymers, by Rich⁶). Figure 2 clearly shows a one-to-one interaction between double-stranded MgDNA and single-stranded NaDNA, in the presence of Mg(II) ion. These experiments were

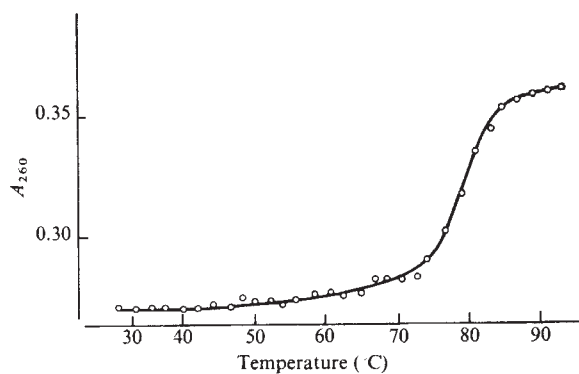
Table 1 Summary of data from continuous variation mixing curves for double-stranded bovine DNA with single-stranded NaDNA

Type of DNA	Added salt	mol % SS NaDNA at A_{260} minimum	DNA ratio at minimum
Doublestrand + singlestrand			
MgDNA + NaDNA	None	No min.	—
MgDNA + NaDNA	0.1 M $MgCl_2$	50%	1:1
NaDNA + NaDNA	SSC	No min.	—
NaDNA + NaDNA	0.1 M $MgCl_2$	No min.	—
MgDNA + Salmon sperm NaDNA	0.1 M $MgCl_2$	No min.	—

repeated with double-stranded NaDNA plus single-stranded NaDNA in the presence of SSC as well as 0.1 M $MgCl_2$. The experimental results are summarised in Table 1, no interaction was apparent in these mixtures. That the reaction may be species specific is indicated in the last experiment reported in Table 1, where denatured salmon sperm NaDNA was substituted for denatured bovine DNA, and no drop in absorbance was observed.

The time required for the formation of the triple-stranded complex was less than 30 s at room temperature. Felsenfeld² found that triple-strand formation took about 24 s with the homopolymers rA and rU in 0.12 M $MgCl_2$.

The amount of triple-stranded bovine DNA formed was estimated by the drop in absorbance at 260 nm (Fig. 2) as compared with the values reported for homopolymers. If we assume that the homopolymers formed a 100% triple-stranded complex, then our DNA was between 15% and 20% triple-stranded. We attempted to separate the various species of DNA (single-stranded NaDNA, double-stranded MgDNA, and triple-stranded) present in the one to one denatured NaDNA and native MgDNA mixture in 0.1 M $MgCl_2$, by sucrose density gradient centrifugation and by CsCl density gradient centrifugation. In all such attempts, we found only one peak caused by DNA, which indicated one species of DNA in the solution. The CsCl₂ buoyant density of this DNA was

**Fig. 3** Temperature-absorbance curve for pure triple-stranded bovine DNA in 0.1 M $MgCl_2$.

1.681 g cm⁻³, when compared with the buoyant density of native DNA (1.699 g cm⁻³). A single species of DNA, resulting from the above mixture, can be visualised as a core of double-stranded MgDNA, to which is attached single-stranded DNA to form short segments of triple-stranded DNA, with many dangling tails of single-stranded NaDNA, and many areas of MgDNA that are solely double-stranded.

To determine the true buoyant density and melting curve of pure triple-stranded DNA, it was necessary to remove all single-stranded and double-stranded segments from the crude triplex. This was done by exhaustive digestion of the triplex mixture with pancreatic DNase I (2 µg ml⁻¹ at pH 6.8) until

no further increase in absorbance at 260 nm was observed. The minimum time required was 4 h. The digest was then separated by gel filtration through a column of Sephadex G-200, 40 × 1 cm, pretreated with 0.10 M $MgCl_2$, which was also used as the eluant. Unhydrolysed DNA was eluted immediately after the void volume and accounted for 18% of the applied A_{260} units. The balance of the 260 nm absorbing material appeared in a complex peak about ten 2.5-ml fractions later, which corresponded well to the elution volume of a mixture of known deoxyribonucleotides. The fractions containing the DNA were pooled, and then concentrated by addition of dry Sephadex G-200. After swelling and water regain had occurred, the mixture was filtered with suction. This final filtrate contained DNA which was resistant to DNase I digestion, that is triple-stranded DNA, and was used to determine the CsCl buoyant density of the pure triplex. Triple-stranded DNA had a buoyant density of 1.726 g cm⁻³ compared with double-stranded NaDNA and MgDNA (both were 1.699 g cm⁻³) and agrees with the values reported for triple-stranded homopolymers^{3,7,8}.

The temperature-absorbance curve for the pure triple-stranded DNA fragments in 0.1 M $MgCl_2$ is shown in Fig. 3. The T_m was 79 °C and the hyperchromicity was 33%. The T_m and hyperchromicities of our three DNAs are compared in Table 2. Since the melting curve of the triplex produced a single break, the three strands seem to be bound equally and are quite stable in 0.1 M $MgCl_2$.

Table 2 Comparison of melting temperatures (T_m) and hyperchromicities of single-stranded, double-stranded and triple-stranded DNA

DNA	T_m (°C)	Hyperchromicity (%)
Denatured NaDNA*	65	13.5
Native MgDNA†	86	43
Triple-stranded DNA*	79	33

* In 0.1 M $MgCl_2$.

† In deionised water.

The biological significance of triple-stranded DNA can only be speculated upon, but it is valuable as a model of possible DNA:RNA triple-strand formation during transcription. Zubay⁹ postulated such a triple strand and concluded that its formation required a widening of the major groove of the DNA. A short triple-stranded DNA segment has also been proposed as an intermediate in DNA replication^{10,11}. The fact that MgDNA was required for triple-strand formation may be explained by the effect of Mg(II) on the DNA helical structure; in contrast to Na⁺, Mg(II) would be expected to bring the phosphate moieties of the opposing strands closer together; thus the major groove of the DNA helix would be widened and triple-strand formation would be possible.

L. E. PERLGUT
D. L. BYERS
R. S. JOPE
V. KHAMVINWATHNA

Department of Chemistry,
California State University,
Long Beach, California 90840

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reviews

ACCORDING to the foreword, the course on which this book is based aimed at providing both a comprehensive survey of basic topics and a review of some more specialised topics of great current interest and intrinsic importance. On that criterion it fails. For example, I find it hard to find the great current interest or intrinsic importance in a three page contribution on rocket soundings in Poland. But then, I'm not Polish.

Had this book been the proceedings of a conference of atmospheric specialists then its contents could not have been faulted, but on the basis of the stated aim I have a vision of eager young workers, new to aeronomy, lusty for understandable and interesting surveys. What they will receive is a set of 16 uncoordinated and rather specialised lectures that present an unbalanced view of the upper atmosphere.

I graded the articles for interest and clarity by pretending that I was a first year graduate student. The results:

- α : Tides (Lindzen), Electrical Structure (Webb), Rockets and Remote Sensing (Heath *et al.*), Lower Ionosphere Morphology (Harnismacher), Winds and Turbulence (Muller), The F Region (Rishbeth).
- β : Photochemical Models (Hesstvedt), D Region Measuring Techniques (Rumi), Composition Studies (Van Zahn), Corpuscular Effects (Mariani).
- γ : The other six articles.

The γ s account for only 50 pages, but their omission would have im-

Air text

Tom Beer

Structure and Dynamics of the Upper Atmosphere. (Proceedings of the Second Course of the International School of Atmospheric Physics.) (Developments in Atmospheric Science, 1.) Edited by Franco Verniani. Pp. xiii+535. (Elsevier Scientific: Amsterdam, Oxford and New York, 1974.) Dfl. 160; \$61.50.

proved the book. They are low quality research contributions that should have been sent to the appropriate research journals. It is a mockery to have a four page article grandiosely entitled "Mid-Latitude Sporadic E" (Bossolasco and Elena); it fails to do credit to an exceedingly important subject.

Had I graded the contributions in terms of their completeness then almost all of them would have been relegated to the β s and γ s. We are presented with 40 pages on winds and turbulence in the meteor zone without the merest whisper of sporadic E (E_s). Harnismacher, to his credit, includes E_s morphology in his excellent contribution but it would come as a surprise to Verniani's scholars at Erice that theories actually exist to account for E_s . They would have left the school, after 535 pages of heavy monologue, unaware of spread F, polar cap absorption, the plasmopause, the polar wind, incoherent scatter radars, the acoustic

cutoff frequency, aurorae and the details of the dynamo current.

Overall, I think the editing was lamentable. The greatest omission was of an introductory chapter that defined a few things. Like: upper atmosphere, ionosphere, stratosphere, D region, and so on. Also, the book is a very uneven mix between theory and experiment. Some topics (such as tides) are only dealt with theoretically, and only the experimental details of others (for example, lower ionosphere) are presented. To complete the confusion, half of the articles use c.g.s. units whereas the other half use m.k.s.

On the credit side, the editor has carefully systematised everybody's bibliographies; the author index and subject index are well prepared; there are surprisingly few typographical errors, and Elsevier have done a fine job on the printing and binding. I unintentionally dropped the book from the back of my speeding motor scooter, an event which the binding has uncomplainingly accepted.

I would only recommend this book to those already actively engaged in work on the upper atmosphere. A few of the articles are excellent, though the active worker will have seen much of the material before in one form or another. Aeronomy desperately needs a lot less edited collections of unrelated papers and a few more single or co-authored works of simple exposition and of authority. I hope that *Developments in Atmospheric Science*, 2 provides the latter. □

THIS book would make a good present for the serious aquarist and would be a useful addition to any departmental library which gives a thought to fostering an interest in freshwater fish and their care. It is not a new book, but a new edition of an old one. An earlier issue was reviewed by Phillip Greenwood in these pages (*Nature*, 198, 516; 1962) and he found much good in it. I concur with his view.

The new edition is compact, strongly bound, and presented as two volumes. The bulk of the text comprises descriptions of the species grouped by families. Each description contains general information at the family level, with distribution charts; and the accounts of the species include more detailed material, biometric data, and illustrations. For the aquarist's special needs information as to behaviour, food

preferences, and environmental requirements with special reference to breeding, are also given.

Some changes have been made in the

Fish out of date

F. R. Harden Jones

Freshwater Fishes of the World. By Gunther Sterba. Vol 1: pp. 1-456; vol 2: pp. 457-877; 191 plates. (Studio Vista: London, 1974.) £12.50.

new edition. Illustrations that were coloured in previous editions are here in black and white, and a whole set of new colour photographs have been included: they are good. The page size has been somewhat reduced and although this makes the book more compact, there is the disadvantage that

drawings originally scaled to natural size are now 10% too small.

And what of the book's deficiencies? These must be measured against one's expectations; I would set those out as 'identification', 'classification', and 'description'. The book fails to clear only the first of these hurdles in that there is no key reference to families, for as Denys Tucker—translator and reviser—notes, that section was still-born. A pity, because it would have put the finishing touches to what is a useful work. There are some trivial mistakes—what book is free from these?—and I would be disappointed to learn that they had been carried through from earlier editions. And no changes have been made to the select bibliography, in which the most recent publication is dated 1962; for £12.50 a purchaser could expect to be taken into the 1970s.

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Allelopathic response

Allelopathy. (Physiological Ecology: A Series of Monographs, Texts and Treatises.) By Elroy L. Rice. Pp. x+353. (Academic: London and New York, September 1974.) \$25.00; £12.00.

MANY people are familiar with the interactions between microorganisms which involve the release of chemical toxins, yet the general public and even many biologists are probably unaware of the importance of such toxic substances in interactions between higher plants. But the concept is by no means new. De Candolle was perhaps the first biologist to suggest such a possibility in 1832. Yet, in spite of that, this book can justifiably claim to be the first volume in English to be devoted to the subject. That alone serves to illustrate the neglect which the study of allelopathy has suffered; it may also be regarded as an indication of the difficulties and uncertainties which surround such a study.

The definition of allelopathy used in the book is that of Molisch, which includes chemical interactions between plant and plant and between microorganism and plant. The study of chemical interactions between plants and animals (included in the term 'allelochemics' as used by R. H. Whittaker) is thus excluded. The emphasis of the book is ecological, and the role of allelopathy in the development of community structure and composition forms a theme linking several sections, whether dealing with phytoplankton, weeds in an old field succession, fire cycles or vegetation pattern in stable communities.

Two chapters on the role of allelopathy in agriculture and horticulture deal briefly but effectively with the considerable volume of work which has been done on this subject. A further section is devoted to the chemistry, physiology and mode of action of the inhibitors involved in allelopathy. The final chapter on chemical reactions between animals and plants is rather incongruous. It is too brief to do justice to a fascinating aspect of plant toxicity and since it does not fall within the definition of allelopathy used by the author, it hardly seems necessary.

Overall, however, the coverage is good and the bibliography excellent. In emphasis and treatment this is essentially a personal view of the subject by Elroy Price. He is well known for his work and ideas on the inhibition of bacterial processes—such as nitrification—during the development of stable vegetation, and on the role of allelopathy in delaying succession in some instances by the attainment of dominance by species with inhibitory properties. These subjects and others like them are developed fully in this book.

At times readability and flow are sacrificed for the author's concern to give practical details (even to the grade of Whatman filter paper used in experiments), but summary paragraphs at the end of chapters make up for this.

I cannot agree with the author's concern to divorce the concept of allelopathy from that of competition. The plant which has evolved allelopathic properties is comparable with a species of coarse or robust morphology in that it is able to substitute space for a resource in competitive interactions. On acquiring such space the resource may become freely available to the plant which would not necessarily require any degree of efficiency in tapping it. To say that the struggle for space is not competition is, in my opinion, an exercise in semantics.

This book is a full and stimulating account of a sadly neglected subject; as such it should be read by all practising ecologists, physiologists, agriculturalists and foresters. It is to be hoped that its production will give impetus to further research in an area where many problems, both theoretical and practical, have yet to be solved. **Peter D. Moore**

Nuclear spectroscopy

Nuclear Spectroscopy and Reactions: vols 40-A and 40-B. Edited by Joseph Cerny. Part A, pp. xvii+518; part B, pp. xvii+711. (Academic: New York and London, October 1974.) Part A: \$44.50; £21.35. Part B: \$49.50; £23.75.

THE rate of growth of the physical sciences is nowadays so great that even the specialist has difficulty in keeping abreast of more than a very narrow field. Yet he needs to know more than this, so it is very important that specialists should periodically summarise the work in their field for the benefit of those working in related areas.

Professor Cerny has succeeded admirably in bringing together the work of a group of well known specialists in nuclear spectroscopy and reactions, and has arranged it in four volumes.

The authors of the articles in the first two volumes—covering nuclear instrumentation and research in nuclear spectroscopy—are among the foremost authorities on their subjects and in many cases are leading the most active groups in the field. The result is most impressive and contains within a relatively manageable compass expert and up-to-date coverage of many of the important growth areas in nuclear spectroscopy. The volumes will be essential reading for all engaged on work in this field. The subsequent volumes, on gamma-ray spectroscopy and theoretical analysis, will be eagerly awaited. **P. E. Hodgson**